Advancing Transplantation

Wadström, Jonas; Ericzon, Bo-Göran; Halloran, Philip F; Bechstein, Wolf O; Opelz, Gerhard; Serón, Daniel; Grinyó, Josep; Loupy, Alexandre; Kuypers, Dirk; Mariat, Christophe; Clancy, Marc; Jardine, Alan G; Guirado, Lluís; Fellström, Bengt; O'Grady, John; Pirenne, Jacques; O'Leary, Jacqueline G; Aluvihare, Varuna; Trunečka, Pavel; Baccarani, Umberto

DOI:
10.1097/TP.0000000000001563

OPEN

Advancing Transplantation: New Questions, New Possibilities in Kidney and Liver Transplantation

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(*Transplantation* 2017;101: S1–S41)

Received 11 August 2016. Revision received 13 September 2016.

Accepted 3 October 2016.

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Astellas job code: ADV/16/0009/EU(2). Date of preparation: March 2017.

Disclosure and contributions: This supplement collects a number of the sessions from the meeting 'Advancing Transplantation: New Questions, New Possibilities'. The meeting was sponsored by Astellas Pharma Europe Ltd; the agenda was developed by Astellas in collaboration with the meeting's scientific committee: J Wadström, BG Ericzon, WO Bechstein, D Serón and PF Halloran. The event was approved by the Federation of the Royal Colleges of Physicians of the United Kingdom for 12 category 1 (external) CPD credits. The scientific committee and faculty developed their own content for the meeting with editorial support from iS Health Group. Editorial support for the meeting was funded by Astellas Pharma Europe Ltd. Previously unpublished data that could not be included, due to existing embargo policies or to protect intellectual property, have been excluded from this report. The unpublished data in this report were included at the discretion of the authors as personal communications.

Based on the presentations given at the meeting and under the direction of the authors, iS LifeScience provided editorial support throughout the development of this supplement. Editorial support was funded by Astellas Pharma Europe Ltd. A.L., in his role as the Guest Editor, reviewed this supplement and advised on the content throughout the development process. The authors had final authority over the editorial content and approved the final version of this supplement before submission.

Astellas Pharma and associated companies developed, manufacture and supply tacrolimus (tacrolimus hard capsules (Prograf), tacrolimus prolonged-release hard capsules (Advagraf)). Prescribing information and adverse event reporting information can be found on pages S40–S41.

J.W. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support and personal fees from Astellas, outside of the submitted work. B-G.E reports nonfinancial support from Astellas, during the development of this supplement; honoraria and consultancy fees from Astellas, Pfizer and Novartis and clinical trial support from Novartis and Astellas, outside of the submitted work. P.H. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support and personal fees from Astellas, outside of the submitted work; shares in TSI, a university company with an interest in molecular diagnostics. W.B. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support and personal fees from Astellas, outside of the submitted work. G.O. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support and personal fees from Astellas, outside of the submitted work. D.S. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support and personal fees from Astellas, outside of the submitted work. J.G. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support and personal fees from Astellas, outside of the submitted work. A.L. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support and personal fees from Astellas, outside of the submitted work. D.K. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support and personal fees from Astellas, outside of the submitted work. C.M. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support and personal fees from Astellas, outside of the submitted work. M.C. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support and personal fees from Astellas, outside of the submitted work. A.J. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support and personal fees from Astellas, Opsona Therapeutics, AstraZeneca, Bayer and Pfizer, outside the submitted work. L.G. reports personal fees from Astellas during the conduct of the study for acting as a conference speaker; personal fees from Astellas, personal fees from Novartis, personal fees from Pfizer, personal fees from Roche, outside the submitted work. B.F. reports nonfinancial support from

Astellas, during the development of this supplement; nonfinancial support and personal fees from Astellas, grants from BMS, grants and consulting fees from Pharmalink, and lecturing fees from Sandoz, outside of the submitted work. J.O.G. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support and personal fees from Astellas, outside of the submitted work. J.P. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support and personal fees from Astellas, outside of the submitted work. J.O.L. reports nonfinancial support from Astellas, during the development this supplement; nonfinancial support and personal fees from Astellas, personal fees from Novartis, and grants from Fisher Scientific, outside of the submitted work. V.A. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support and personal fees from Astellas, outside of the submitted work. P.T. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support and personal fees from Astellas, outside of the submitted work. U.B. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support from and personal fees from Astellas, outside the submitted work. J.N. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support and personal fees from Astellas, and personal fees from Novartis, outside of the submitted work; employment as a consultant physician at Queen Elizabeth Hospital, Birmingham. A.S.-G. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support and personal fees from Astellas, outside of the submitted work. E.G. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support from Astellas, and personal fees from Astellas, Pfizer and Novartis, outside the submitted work. M.M. reports nonfinancial support from Astellas, during the development of this supplement; nonfinancial support and personal fees from Astellas, outside of the submitted work. M.G. reports nonfinancial support from Astellas during the development of this supplement; nonfinancial support from Astellas and personal fees from Astellas, outside the submitted work.

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ISSN: 0041-1337/17/10102-S1

DOI: 10.1097/TP.0000000000001563

INTRODUCTION

Associate Prof Jonas Wadström and Prof Bo-Goran Ericzon

This supplement reports on the proceedings of a meeting titled "Advancing Transplantation: New Questions, New Possibilities," held at Karolinska Institutet in Stockholm, Sweden, on January 24 to 26, 2015, and sponsored by Astellas Pharma Europe Ltd. The meeting highlighted the challenges facing the transplant community and the need to respond to those challenges with new approaches, questions and possibilities. Over 450 kidney and liver transplant professionals from across Europe attended the meeting, which included talks from keynote speakers, scientific presentations, panel discussions, interactive sessions, and a poster session designed to allow delegates to exhibit their own clinic's data.

Solid organ transplantation has evolved into one of the great accomplishments in clinical medicine and remains the only lifesaving treatment for many types of end-stage organ failure. Breakthroughs in transplant procedures and the development of effective immunosuppressive therapies have helped health care professionals achieve significant improvements in graft and patient survival posttransplant. This is evident across all indications, including kidney and liver transplantation.[1,2] Continuing the advances in improving long-term survival remains a key challenge for transplant medicine today.[2,3]

Maintaining a transplanted graft over time is complex. Multiple risk factors influence graft survival before, immediately after and late after transplantation. The use of marginal donors, to reduce the disparity between demand and availability of organs, has added to this complexity and brought new challenges to the field of transplantation. Recent research in the kidney and liver transplant arenas has identified a number of risk factors that contribute to poor graft survival (Figure 1). These risk factors can lead to irreversible pathological damage to the transplanted organ, with a negative impact on patient outcomes. By managing these risk factors, we aim to improve the long-term survival of transplanted grafts for our patients.

Health care professionals need to continually reassess how to improve care for their patients while managing limited resources and embracing the new age of digital technology and big data. Only by understanding the risk factors that are modifiable and translating this into changes in the clinic will the perspectives for patients be further improved. "Best practice" care for transplant patients needs to be constantly updated, taking new clinical developments into consideration. Here, we present our findings with regard to the ongoing risk factors for poor long-term outcomes in kidney and liver transplantation and our suggestions for best-practice management for each of these risk factors. We also include speculations as to future innovations that have the potential to change patient management for the transplantation and wider health care communities.

- Improving long-term graft and patient survival remain the key challenges for transplant medicine today; maintaining a graft over time is complex with multiple risk factors that influence graft survival from organ procurement to posttransplant follow up
- To improve graft and patient survival, both traditional and emerging risk factors need to be identified and modified, including nonadherence to treatment, high variability of calcineurin inhibitor (CNI) exposure and underimmunosuppression or overimmunosuppression
- Only by identifying these risk factors early and monitoring for them routinely in clinical practice, will the patients at risk of poor long-term outcomes be determined
- A number of risk factors for poor graft and patient survival can be modified by the choice of immunosuppressive regimen used

PART 1: ONGOING CHALLENGES WITHIN KIDNEY AND LIVER TRANSPLANTATION

Beyond Randomized Controlled Trials: Looking at Data Differently

Dr Philip F Halloran

A key unmet need currently facing organ transplantation is the requirement to improve transplant management in an era where there are few new pharmaceutical company-sponsored phase II and III clinical trials. To achieve this, it will be necessary to optimize the use of new data sources to understand outcomes, classify disease states, and confirm

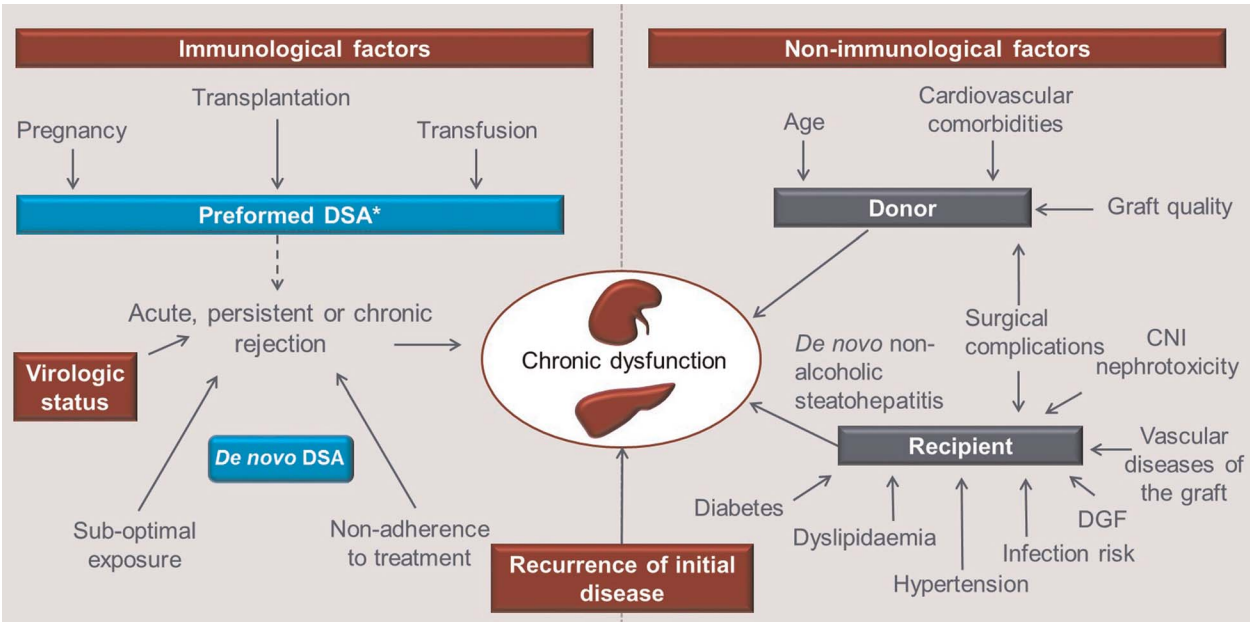


FIGURE 1. Risk factors associated with poor graft survival in kidney and liver transplantation. *Fortunately, most liver transplant patients with preformed DSA have uneventful resolution posttransplant; even liver patients with class II DSA with mean fluorescence intensity >10 000 [LABScreen single-antigen beads (One Lambda)] experience resolution two-thirds of the time.[4] CNI, calcineurin inhibitor; DGF, delayed graft function; DSA, donor-specific antibody.

associations in prospective, investigator-sponsored, observational trials at the individual patient level. This new understanding could lead to future tests, interventions, and clinical trials, supported by the industrial investment that is needed for optimal progress.

Randomized controlled trials (RCTs) have facilitated many improvements in transplantation and continue to represent the standard we should strive to achieve. However, as a data source, the RCT has some significant disadvantages, particularly the inclusion of highly selective populations that may not adequately reflect the real world. These populations are often excessively 'front-end loaded', being recruited at the time of transplantation, with most events occurring in the first few months posttransplantation, yet they are being used to develop principles for long-term management. To put this into perspective, most of the evidence from trials has been based on events in the first year, yet 90% of organ transplant patients are living beyond their first year posttransplant. The protocols used can also affect the suitability of the data, including the use of intent-to-treat designs, limited study durations, and little human leukocyte antigen (HLA) antibody phenotyping or recognition of antibody-mediated rejection (AMR). RCTs are more likely to include adherent patients, underrepresenting the serious problem of nonadherence and difficulty in paying for the drugs long term.

Registries have several advantages over RCTs in that their data sets are larger, all-inclusive, more diverse, and a better representation of the real world, producing more generalizable conclusions. Registry data, however, can also be heavily influenced by inaccurate and incomplete data and confounders that lead to inappropriate conclusions. Registries suffer from poor phenotyping, often permitting meaningless categories, such as "chronic rejection." Within these limitations, registries remain important sources of data and should not be underestimated. They are most useful for studying unequivocal variables such as age and endpoints, such as patient and graft survivals, and they have been useful in confirming the results of phase III trials. However, it is important to distinguish between the trends that may be revealed by the associations, and causality.

An example of the complexities of interpreting associations within registries can be seen in the association of donor age and impaired graft survival. One of the driving factors of the deceased donor-risk index is the age of the donor.[5] For example, as the age of the donor and the risk index increase, there is a stepwise decrease in liver survival.[5,6] The key question is to understand the basis of this association and the phenotype and mechanism of the failures. Associations with donor age are complicated by the inherent bias in organ allocation. Because of clinician reluctance to give older organs to low-risk young recipients, old organs are allocated preferentially to high-risk older recipients, often with serious comorbidities that are difficult to capture in databases. This strongly links the influence of old donor age and old recipient risks, a bias that is not readily corrected by statistical methods.[7] In kidney transplantation, the relationship between recipient age and donor age is so strong that it is often impossible to ascribe a phenotype to either variable alone.[7] One result could be to overestimate the adverse effects of donor age, triggering unnecessary organ discards. The solution to this is to always try to translate risk factors into phenotypes and mechanisms, and look for ways in which an association may be misleading because of confounders. In other words, try to explain individual failures in actual patients.

Improper analysis and incorrect validation have often occurred in the application to transplantation of microarrays and other "omics" technologies producing high-dimensionality data, that is, many measurements per sample. Such data have previously been criticized for "noise discovery," particularly in cancer research.[8] However, a strict systematic approach can harness these technologies and avoid such errors,[9] and

can make microarrays and other molecular methods suitable for diagnostic discovery and applications. Our strategy has been to develop a reference set of biopsies and the patients they represent, establishing the key parameters of the phenotype with maximum granularity and including clinical, laboratory, histologic and molecular data. The errors in the conventional classification should be corrected; the conventional phenotype can then be used to "train" diagnostic tests using the molecular measurements derived from the microarrays, assigning a molecular class to each biopsy by a diagnostic equation (classifier) derived through machine learning, using methods such as cross-validation to prevent overfitting. The results should be validated in an independent biopsy set. Once conventional classes have trained molecular tests, the molecular tests can be used as standards to refine the conventional classes, forming an iterative loop. It was this iterative strategy that led us to realize that AMR was being greatly underestimated by the requirement for C4d positivity; we established the molecular phenotype then realized that the molecular changes of AMR were much more common than C4d staining.[10,11]

Some of the older phase III clinical trials and registry data analyses need to be reinterpreted as we learn about the limitations of the diagnosis of rejection in earlier eras. This is reflected in a recent study, which showed that T cell-mediated rejection (TCMR) in indication biopsies does not impact survival compared with the absence of rejection within an indication biopsy population,[12] contrasting with the devastating effect of AMR in that population.[12,13] The limitation is that the best group to be in—those who never have indications for biopsy—is not in the population studied. Another study showed that v-lesions (intimal arteritis) are now less common and milder (usually v1), and have little effect on prognosis except to the extent that they reflect AMR.[14] Such analyses challenge the conventional wisdom of the significance of "rejection" and v-lesions in diagnosis and prognosis. This illustrates the problem of "data drift"; that is, associations and risks change as practice changes. Conclusions reached in earlier eras must be constantly reassessed for validity and relevance.

Analysis of "big data" from large administrative databases and electronic health records has been used effectively in some areas of medicine and may have applications in transplantation, provided the same critical standards are applied and confounders are sought. The predictions from such studies must be validated in prospective studies, and the mechanisms of the associations and the disease phenotypes should be understood at the level of the individual patients.

In conclusion, there are many opportunities beyond phase III trials and much of the new insights will come from such opportunities in the next decade. Research should start and end with the struggle to understand and predict the events in the individual patient, coupled with a questioning attitude to the conclusions from earlier eras and a critical examination of the literature. Whether using registry data, high-dimensionality data, or phase III trials, the project must be approached with rigorous designs and validation, and must search for mechanisms that explain associations. The goal must always be to explain actual phenotypes and outcomes in the clinic, and to use that information to change care. This, in turn, will create opportunities for investment.

Optimizing Immunosuppression: What are the Registries Telling Us? The European Liver Transplant Registry

Prof Dr Wolf O Bechstein

As we enter an era in transplantation where there are fewer ongoing large RCTs, we also rely on alternative methods of data collection and analysis to answer clinical questions in an attempt to further improve the long-term outcomes of our patients. Not only do registries provide us with a large amount of data that continues to expand over time, they also provide long-term follow up beyond that of RCTs.

The European Liver Transplant Registry (ELTR) was inspired by the vision of Henri Bismuth, and was established to create a database comprising the whole experience of European liver transplant centers. Data are collected prospectively using a standardized 2-part questionnaire,[15] which is regularly updated by a scientific committee. Part 1 comprises date and indication for the liver transplantation, donor and recipient data, surgical technique used and immediate postoperative immunosuppression therapy, and Part 2 comprises graft and patient outcomes and the longer-term immunosuppressive regimen. The data held in the registry are subject to strict internal and external quality control, with annual audits of randomly selected centers.[15] In 2003, an independent audit of 21 centers found that 95% of the data held in the ELTR was complete and the rate of consistency between the data held by the hospitals versus the ELTR was 98.5%.[16] These findings indicate a high degree of accuracy for the data held in the registry. To our knowledge, this is the only transplant registry with regular random-sample external auditing. Data from the ELTR have provided valuable insights in liver transplantation, including information regarding the use of organs from marginal donors,[15] and the development of a risk-analysis model to determine mortality risk of a given procedure in an individual patient.[17]

Adam et al[18] recently reported an analysis of data from the ELTR comparing long-term graft and patient survival data for all adult patients transplanted between January 2008 and December 2012 who received tacrolimus-based immunotherapy. The patient groups were stratified by the tacrolimus formulation they received during the first month posttransplant (prolonged-release vs immediate-release formulation). For further analysis, patients remained in the originally assigned group even if the formulation was changed, similar to an intent-to-treat analysis in RCTs. Data for the patients with <1 month of follow-up were excluded. To prevent center bias, only data from the 21 centers who had been using both tacrolimus formulations were included.[18] Data for 4367 patients were analyzed (528 patients receiving prolonged-release tacrolimus and 3839 patients receiving immediate-release tacrolimus).[18] In this study, the group of patients receiving prolonged-release tacrolimus demonstrated a significantly higher rate of graft survival compared with the group receiving immediate-release tacrolimus over 3 years of treatment (88% vs 80%, respectively; $P = 0.01$). The numeric difference of 8% between the treatment arms amounts to a 10% improvement in long-term graft function, or, in other terms, a 40% risk reduction of graft loss.

The 3-year patient survival was 88% in the prolonged-release versus 82% in the immediate-release tacrolimus group ($P = 0.07$). The other risk factors reported included recipient dialysis, higher United Network for Organ Sharing (UNOS) status and model for end-stage liver disease (MELD)

score, serum creatinine ≥2 mg/dL, total ischemia time ≥12 hours, and hepatocellular carcinoma (HCC), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) status. In the multivariate analysis, the 3 highest-ranking risk factors for reduced graft and patient survival were found to be HIV-positive serology (risk ratio: 3.40, 3.41, respectively), serum creatinine concentration of ≥2 mg/dL (risk ratio: 1.84, 1.86, respectively), and treatment with immediate-release tacrolimus immunotherapy versus prolonged-release tacrolimus (risk ratio: 1.81, 1.72, respectively) (Table 1). Adam et al[18] went on to report 3-year Kaplan–Meier data showing an 8% graft survival advantage in the group receiving prolonged-release tacrolimus versus those in the immediate-release tacrolimus group (P = 0.01) (Figure 2) and a 6% (nonstatistically significant) trend toward improved patient survival in the group receiving prolonged-release tacrolimus versus those in the immediate-release tacrolimus group (P = 0.07). Longer-term follow-up data for patients in the ELTR and an analysis of patients who switched between formulations after month 1 are ongoing.

Adam et al[18] carried out further analyses on a refined population to account for discrepancies in the baseline characteristics between the 2 treatment groups using propensity score matching (where patients were paired according to similar predefined baseline characteristics, which had been identified as significant risk factors in previously carried out univariate and multivariate risk analyses) on a 1:2 ratio (prolonged-release tacrolimus:immediate-release tacrolimus). When the authors analyzed data from these 810 patients (270 prolonged-release, 540 immediate-release tacrolimus) they confirmed a significant graft survival advantage associated with the use of prolonged-release versus immediate-release tacrolimus over 3 years in both the univariate and Kaplan–Meier analyses. The patient survival advantages also reached statistical significance. In the multivariate analysis of the propensity score matched patient cohorts, immediate-release tacrolimus immunotherapy was confirmed as a risk factor for reduced graft and patient survival, second only to ABO blood group incompatibility.

As we are beginning to understand more about the modifiable risk factors in transplantation, including nonadherence, variability of tacrolimus exposure and underimmunosuppression, this will help us to further understand the causes of the differences seen with prolonged-release compared with immediate-release tacrolimus in terms of graft and patient survival.

In conclusion, these data show significant benefits in survival postliver transplantation in patients receiving prolonged-release versus immediate-release tacrolimus; however, a better understanding of the reasons for the differences in survival is needed. This is an exciting era, but our knowledge must be transferred into clinical practice for these survival advantages to be achieved in larger patient populations, and we therefore need to redefine best practice for our patients. It is important to establish how we can "operationalize" these data to improve clinical practice.

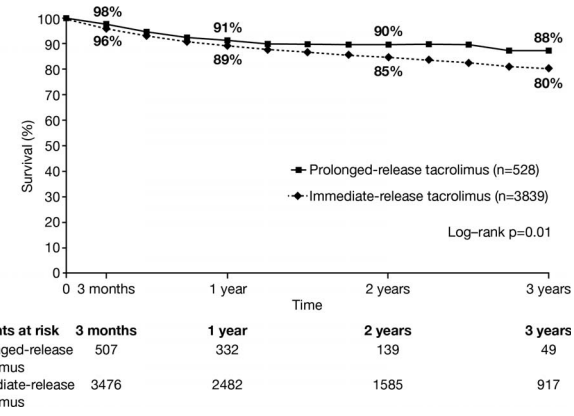


FIGURE 2. Kaplan–Meier analysis of graft survival over 3 years of treatment with prolonged-release tacrolimus compared with immediate-release tacrolimus after exclusion of patients with <1 month of follow-up. Reprinted from Adam R, Karam V, Delvart V, et al. Improved survival in liver transplant recipients receiving prolonged-release tacrolimus in the European Liver Transplant Registry. *Am J Transplant.* 2015;15:1267–21282 doi:10.1111/ajt.13171.

Optimizing Immunosuppression: What are the Registries Telling Us? The Collaborative Transplant Study

Prof Gerhard Opelz

The Collaborative Transplant Study (CTS) was initiated in 1982, and over the last 30 years, has collected a wealth of data on kidney, pancreas, heart, lung, and liver transplants that have helped shape clinical practice. This registry was built on a philosophy that the knowledge-gaining process can be accelerated by combining the experiences of many, especially with respect to complex interactions of factors and the analysis of rare events.[19] In a therapy area where there are fewer RCTs, and in an era of big data, the transplant community is beginning to recognize the increasing importance of registry data to inform decision making that affects patient care and access to medicine.

The CTS is a rigorous and strictly scientific registry based on voluntary participation; over 20 000 new patient data sets

TABLE 1.

Multivariate analysis of risk factors for reduced graft survival after exclusion of patients with <1 month of follow-up

Risk factors at first transplant	Risk ratio	95% CI	*P*
Recipient HIV-positive	3.40	2.04-5.68	<0.0001
Serum creatinine concentration ≥2 mg/dL	1.84	1.42-2.39	<0.0001
Immediate-release tacrolimus immunotherapy	1.81	1.26-2.61	0.001
UNOS status 1 or 2	1.61	1.30-2.00	<0.0001
Recipient anti-HCV positive	1.51	1.24-1.83	<0.0001
Total ischemia time ≥12 h during first liver transplant	1.42	1.06-1.89	0.02
Recipient age ≥50 y	1.41	1.15-1.73	0.001
HCC (primary or secondary disease)	1.37	1.11-1.67	0.003
Donor age ≥50 y	1.33	1.10-1.60	0.003

N = 3828. CI, confidence interval; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; UNOS, United Network for Organ Sharing. Table from Adam et al.[18]

are registered each year, the majority of which are in kidney transplantation.[1] Today, there are approximately 480 transplant centers participating in the CTS: 292 specializing in kidney transplantation, 87 in liver transplantation, and 101 in heart and lung transplantation. Although most of the centers originate in Europe, participation is worldwide and includes representation from South and North America, Asia, and Africa. The last 30 years has seen improvements in short-term and long-term survival rates across all transplant fields, owing to significant advances in transplant procedures and immunosuppressive therapies.

Clinical practice is continuously evolving with the addition of new data. Focusing on the kidney transplant setting, in 1985, data from the CTS showed a significant improvement in outcomes with cyclosporine A (CsA) compared with existing immunosuppressive regimens.[20] Over the next decades, we reported that discontinuing steroid therapy was associated with improved long-term graft and patient survival postkidney transplant, as well as a reduction in osteoporosis and cataracts, and a trend toward reduced hypertension.[21-23] Previously, data from the CTS registry also demonstrated a link between maintenance steroid dose and death due to cardiovascular disease or infection, although there was no association with death due to malignancies.[1]

From 2003 onward, there has been a dramatic shift in the treatment paradigm, with the overwhelming majority of patients now receiving tacrolimus-based immunosuppression rather than CsA. Consistent with previous reports, reduction of maintenance steroid dose at 1 year was also shown to minimize the risk of cardiovascular-related and infection-related deaths in patients receiving tacrolimus therapy. Data from the CTS registry also supported results from other studies in demonstrating that tacrolimus minimization 2 years posttransplant is associated with an increased risk of graft loss.[24] One possible explanation is that underimmunosuppression allows the T cell response to return, increasing the production of donor-specific antibodies (DSA) and leading to AMR.

Data from the CTS have shown a gradual, but significant, decrease in median tacrolimus trough levels from 2003 onward (Figure 3).

This led us to question whether the decline in tacrolimus trough levels observed has impacted graft survival. Analysis of graft survival outcomes demonstrated that tacrolimus trough levels less than 5 ng/mL at 1 year posttransplant were significantly associated with inferior graft survival over 6 years (Figure 4). Although the causes of graft loss for all

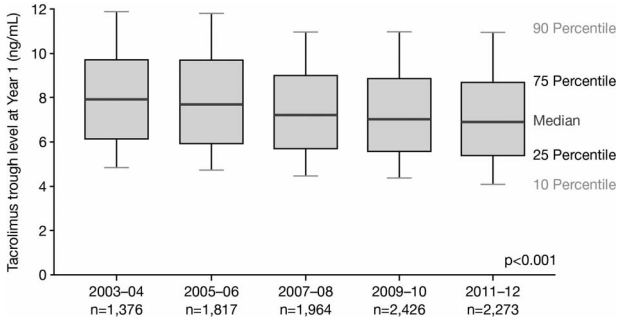


FIGURE 3. Median tacrolimus trough levels at 1 year postkidney transplant from 2003 onward. *P* value calculated using the Jonckheere trend test.

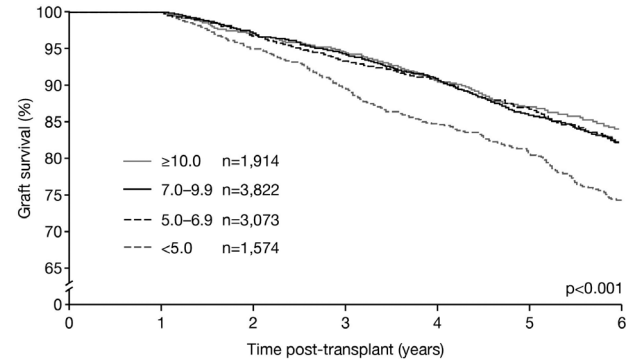


FIGURE 4. Graft survival according to tacrolimus trough levels at year 1 postkidney transplant. *P* value calculated using the log-rank test for trough levels for all comparisons compared with the <5.0 ng/mL group. (Collaborative Transplant Study, written communication, January 25, 2015).

patients are unknown, these data suggest that trough levels less than 5 ng/mL might not provide sufficient immunosuppression postkidney transplantation (Collaborative Transplant Study, written communication, January 25, 2015).

Evaluation of tacrolimus trough levels over time has shown a rise in the proportion of patients with trough levels less than 5 ng/mL (from 10% in 2003 to just under 20% in 2012), presumably in conjunction with a trend for tacrolimus minimization in clinical practice. Furthermore, in patients with tacrolimus trough levels less than 5 ng/mL at 1 year, the dose of mycophenolic acid (MPA) was found to be a critical determinant for graft survival, with low doses of MPA at 1 year (≤1 g/d) being associated with reduced graft survival.[25] However, it would be wrong to conclude that increasing the dose of MPA provides sufficient immunosuppression in patients with low tacrolimus trough levels. In fact, patients receiving higher dose MPA (with tacrolimus trough levels <5 ng/mL at year 1), showed increases in tacrolimus trough levels in subsequent years, presumably as a result of clinical need.[25] This underscores the importance of targeting trough levels greater than 5 ng/mL, regardless of MPA dose.

In patients with good graft function at 1 year, higher tacrolimus trough levels were not associated with an increase in new-onset diabetes after transplant (NODAT) or nephrotoxicity (as evidenced by serum creatinine levels) at 5 years compared with lower tacrolimus trough levels. Interestingly, in patients with impaired graft function, a strong stepwise correlation between graft function at 5 years and high tacrolimus trough levels was observed, indicating that raising tacrolimus trough levels beyond 5 ng/mL improves graft function in these patients.

Data in the CTS registry collected from patients treated with the once-daily, prolonged-release formulation of tacrolimus are currently being analyzed. It will be interesting to assess if the improvements in long-term graft and patient survival with prolonged-release versus immediate-release tacrolimus that have been reported in liver transplantation[18] are also apparent in kidney transplantation.

In conclusion, the CTS registry provides a wealth of real-world data that can inform clinical practice and improve the outlook for kidney transplant patients. Where possible, reduction or withdrawal of corticosteroids should be considered in patients receiving tacrolimus-based therapy to improve

long-term results. Underimmunosuppression with tacrolimus trough levels less than 5 ng/mL at 1 year is also associated with inferior graft survival over the long term. As such, every care should be made to maintain tacrolimus trough levels above this threshold. Contrary to previous beliefs, higher tacrolimus levels are not associated with NODAT and nephrotoxicity in patients with good graft function. The observation that increasing tacrolimus trough levels may improve graft function in patients with impaired graft function is intriguing and warrants further investigation.

As the importance of these types of observational studies becomes increasingly evident, there is an ongoing shift in attitude to see the value of registry data in informing critical decision making. Moving forward, even with the challenges outlined in the previous section, registry data are set to support data generated via RCTs and impact patient management, therapies, research and innovation.

The Kidney Graft Journey and Risk Management

Prof Daniel Serón

The insidious accumulation of risk over time can lead to poor long-term outcomes; we know that the interrelationships between risk factors for graft survival, due to the complexity of the transplanted graft, are complicated. However, some of these risk factors can be modified by making changes to our patient management and clearly defining the best practice of care.

It is important to evaluate and reevaluate the risk factors for poor graft survival as clinical practice changes. Over the last few decades, knowledge, attitudes, pharmacological interventions, concerns about cardiovascular disease, and other factors, have evolved. A Spanish epidemiology study showed that late renal allograft failure is a changing scenario due to changes in patient management. For example, between 1990 and 1998 there was a decline in rejection but an increase in cytomegalovirus infection.[26] The risk factors over time for an individual patient also change; those at 5-year and 10-year posttransplant have different challenges when compared with those at 1 year.

Even our understanding of the effect of risk factors on long-term outcomes at 1 year posttransplant has changed. Ten to 15 years ago, there was a belief that CsA minimization improved and preserved renal function; however, progression of interstitial fibrosis assessed by biopsies at month 4 and year 1 posttransplant were associated with underexposure of immunosuppression.[27] In general, tacrolimus-treated patients have been shown to have a better graft survival rate versus CsA.[28] Furthermore, tacrolimus minimization and withdrawal strategies have been associated with a decline in graft survival compared with tacrolimus continuation.[29] Our understanding now is that to target tacrolimus trough levels too low constitutes a significant risk for poor outcomes. High variability of tacrolimus exposure is also considered to be a modifiable risk factor for kidney transplant recipients and has been associated with an increased risk of rejection and poor graft survival.[30,31]

Another modification of our beliefs has been the assessment of patients who are nonadherent to treatment, and this is continuing to evolve. Fifteen years ago, we believed that most transplant recipients were adherent. In Europe particularly, this seemed logical because the European health systems fund immunosuppressive drugs, meaning that cost is not a barrier to adherence in these patients. However, we now understand that nonadherence is a significant concern in transplantation and data from the United States Renal Data System have confirmed that it is, indeed, a risk factor for poor graft survival.[32]

In order to improve outcomes for our patients, the initial conditions of the transplanted graft need to be assessed, as these have an impact on its long-term survival. Nankivell et al[33] demonstrated that subclinical inflammation posttransplant is a risk factor for progression of chronic interstitial damage. Interstitial fibrosis/tubular atrophy and inflammation together in the same biopsy have also been shown to have an elevated risk effect for poorer long-term outcomes,[34] whereas in another study, inflammation detected in 1-month and 4-month biopsies has been associated with progression of fibrosis at 1 year.[35]

More recently, it has been shown that inflammation early posttransplant is associated with the development of de novo DSA in patients who are nonadherent to treatment.[36] There is also a correlation between high levels of inflammation in the interstitium and elevated donor-specific immune response.[37] Another important modifiable risk factor that occurs early in the graft journey is ischemic reperfusion injury (IRI), which can inflict irreversible damage to the kidney. The inflammation associated with IRI may play a role in the cardiovascular risks associated with transplantation.

Interestingly, these risk factors can be modified by the immunosuppressive regimens used, for example, everolimus has been associated with a higher incidence of DSA versus CsA,[38] while tacrolimus-based regimens are associated with less inflammation compared with CsA-based regimens.[39] With tacrolimus immunosuppression in mind, it seems a fair assumption that good adherence to treatment, low variability and adequate exposure play a role in reducing inflammation, DSA and, subsequently, AMR early posttransplant. Moreso et al[39] demonstrated that baseline immunosuppression not only influences the incidence of clinical acute rejection (AR), it also influences the degree of inflammation in early protocol biopsies. Once we diagnose "full-blown" AMR, our options to modify its natural history and the graft journey are very low; we need to act early, as the decisions we make early posttransplant impact long-term outcomes. Even the risk factors that may not translate to changes in creatinine and proteinuria have important consequences 5 to 10 years posttransplant.

Improving long-term outcomes remains a common goal in transplantation, and we know that immunologic and nonimmunologic risk factors have an impact on graft survival. We need to concentrate our efforts on modifying the risk factors that may improve survival early posttransplant. The therapy that we choose for our patients is a variable that can be modified and there is probably still room to better use these immunosuppressive treatments. More research is needed in both a randomized-controlled setting and in a real-world environment to determine the optimal immunosuppressive regimens for each patient group. The following sections in this paper further discuss a number of modifiable risk factors, including the development of DSAs, underimmunosuppression, nonadherence to treatment, IRI, delayed graft function (DGF) and cardiovascular complications.

Early Ischemic Injury and DGF in Kidney Transplantation

Prof Josep Grinyó

Although IRI is considered to be unavoidable in solid organ transplantation, there are a number of new diagnostic and therapeutic approaches that could help to improve long-term graft outcomes. Organ preservation and management needs to begin at the source; by managing our donors in a better way, improving organ preservation after retrieval and attenuating reperfusion, we can aim to prolong the life of the transplanted graft.

The UNOS previously recommended a variety of predefined donor management goals to optimize the hemodynamic stability of the transplanted graft.[40] Where these strategies have been implemented in clinical practice the risk of DGF in kidney transplantation has been reduced by approximately 50%.[40]

Nowadays, we have to deal with a population of donors with organs that may be more susceptible to IRI; we are an aging society with older recipients and donors. It would be expected that patients who receive organs from expanded-criteria donors (ECDs) would have higher rates of DGF, poorer renal function and reduced graft survival, as they may be more vulnerable to the inflammation generated during IRI. These organs, which potentially have preexisting lesions, could also have an impaired capacity for regeneration after IRI.

Data from the Organ Procurement and Transplantation Network/Scientific Registry of Transplant Recipients support these observations and show that the incidence of DGF is dependent on donor characteristics. For the ECDs who donated after cardiac death, there was an over 50% increase in DGF compared with the standard-criteria donors (SCDs).[41] Similarly, Moers et al[42] reported a higher odds ratio of DGF for ECDs versus SCDs. ECDs were also associated with a higher risk of graft failure within the first year posttransplant.

There is a gap in the literature between experimental research addressing IRI and clinical data that can be translated to clinical practice, although there is ongoing research into modifying risk factors associated with IRI.[43] A US registry analysis demonstrated that machine perfusion reduces the risk of DGF and 1-year graft failure by approximately 50% versus cold storage[42]; however, there was no significant difference in the magnitude of the treatment effect on DGF with SCDs versus ECDs.[42] A meta-analysis demonstrated similar results,[44] whereas in a separate study, graft survival remained significantly higher in the machine perfusion group at year 3.[45] Kwiatkowski et al[46] reported a significant improvement in both graft survival and renal function at 5 years. In a separate study, DGF and cold ischemia time more than 24 hours were associated with a reduction in graft survival; however, patients with DGF but without AR did not experience an inferior graft survival rate.[47] Different thermic regional perfusion techniques show promise; when studied in organs donated after circulatory death, normothermic regional perfusion, in particular, has considerable potential to restore the quality of these grafts.[43] Oniscu et al[48] also demonstrated a low occurrence of DGF (40%) when normothermic regional perfusion was used. When looking at clinical practice, we see that machine reperfusion is being used, especially in centers that have ongoing ECD programs. To complement these efforts, there is a Consortium for Organ Preservation in Europe that has been formed to conduct several randomized trials on different techniques for the preservation of kidney and liver transplants. We eagerly await these results.

Donor management strategies should be used within the context of patient management and alongside pharmacological therapies in an attempt to improve patient outcomes. Taking into account the many different pathways and mechanics involved in IRI, by using multitargeted therapy (as we do in the case of the prevention of AR), we could further improve long-term outcomes.

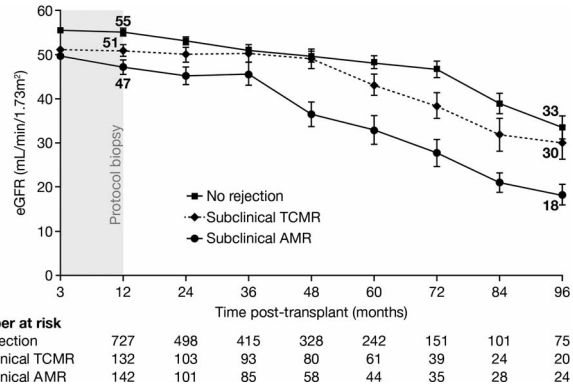
Perhaps the most effective tool that we have available in our clinics today for the preservation of kidney transplants is to assess the risk of developing DGF. Irish et al[49] reported a score index that has now been adopted in a number of phase II trials to predict the high-risk patients who would benefit from selective interventions. These assessments of risk for DGF could be a useful tool in clinical practice to select those patients that may benefit most from the new techniques and pharmaceutical interventions. By identifying at "risk" patients and modifying the risk factors for poor graft survival, we may achieve better outcomes for our patients in the long term.

DSA-Mediated Allograft Injury in Kidney Transplantation: New Understandings

Dr Alexandre Loupy

Over the years, DSAs have become a major challenge in solid organ transplantation and are now the cornerstone of allograft injury in many areas, including renal, heart, pancreatic, liver, and lung transplantation. Because AMR is the leading cause of kidney allograft loss,[50] focusing on improving adherence to immunosuppressive regimens and ensuring adequate exposure to tacrolimus is essential to avoid the formation of de novo DSAs and graft failure.

Advances in assays and screening techniques have helped to highlight the emergence of DSAs and enabled a better understanding of their contribution to events such as AMR. Recent studies have focused on the subclinical AMR outcomes and highlight the necessity of improving the therapeutic management of our patients. In an observational prospective study of 1307 kidney allograft recipients transplanted in Paris between 2000 and 2010, subclinical AMR at 1 year was one of the main independent determinants of long-term kidney allograft loss, independent of conventional assessments.[13] The patients were divided into 3 groups based on their phenotype at 1 year posttransplant: those with no rejection (n = 727), those with subclinical TCMR (n = 132) and those with subclinical AMR (n = 142). Patients with subclinical AMR at 1 year had worse kidney allograft function at 8 years compared with the patients in the subclinical TCMR and nonrejection groups (Figure 5).[13] Additionally, at year 8 posttransplant, patients in the subclinical AMR group had the poorest graft survival (56%) compared with the subclinical TCMR (88%) and nonrejection (90%) groups ($P < 0.001$).[13] Subclinical AMR at 1 year posttransplant was evaluated in a multivariate Cox model and was independently associated with a 3.5-fold increase in long-term allograft loss (95% confidence interval (CI): 2.1-5.7), together with proteinuria and a low or intermediate estimated glomerular filtration rate (eGFR) ($P < 0.001$).[13] These data accentuate the growing concern regarding indolent AMR phenotypes on long-term outcomes. Other studies reinforcing this concern show that,



Number at risk								
No rejection	727	498	415	328	242	151	101	75
Subclinical TCMR	132	103	93	80	61	39	24	20
Subclinical AMR	142	101	85	58	44	35	28	24

FIGURE 5. Long-term kidney allograft function according to a 1-year subclinical rejection profile. The evolution of eGFR (MDRD) in 1001 patients on the basis of assessment of 4 511 longitudinal eGFRs is shown. The long-term course of eGFR in 3 groups of patients was evaluated using a linear mixed model starting from 1-year post-transplant to the last available eGFR; 905 eGFR measurements taken at 3 months posttransplant are also shown. Bars represent SDs. AMR, antibody-mediated rejection; eGFR, estimated glomerular filtration rate; MDRD, modification of diet in renal disease; SD, standard deviation; TCMR, T cell-mediated rejection. Adapted with permission of *The Journal of the American Society of Nephrology* from Loupy A, Vernerey D, Tinel C, et al. Subclinical rejection phenotypes at 1 year post-transplant and outcome of kidney allografts. *J Am Soc Nephrol.* 2015;26:1721–1731. Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

despite a decrease in clinical AMR, the anti-complement component C5 antibody treatment, eculizumab, could not reduce the subclinical forms of AMR.[51] These recent results have reshaped our understanding of AMR, defining it as a continuous process with varying levels of injury and stages rather than a discrete event.

Recently, a number of studies using contemporary tools have increased the knowledge of the pathogenesis of DSAs. In particular, a population-based study of 1016 carefully phenotyped kidney transplant recipients from 2 French centers demonstrated that the complement-binding capacity of DSAs play an important role in kidney allograft failure. Patients with complement-binding DSAs posttransplant had a worse allograft survival rate at 5 years (54%) compared with those with non-complement-binding DSAs (93%) and patients without DSAs (94%; $P < 0.001$ for both comparisons). This study emphasized that the addition of the complement-binding capacity of DSAs to conventional risk factor models should be used to identify patients at high risk for kidney-allograft loss.[52] Further results of an independent analysis examined the impact of immunoglobulin G (IgG) subclasses on injury patterns and suggested that subclinical AMR is typically driven by IgG4, whereas acute clinical AMR is mainly driven by IgG3.[53]

In an attempt to address the limitations of conventional histologic assessment, molecular microscope biopsy measurements, using a microarray-based molecular microscope, have added to the armamentarium for the assessment of transplant biopsies.[54] Molecular microscopy was used in addition to conventional clinical, histologic and immunologic features to evaluate the potential impact of this approach in terms of prediction of the risk of graft loss and disease progression. Adjusting for conventional features, AMR molecular score (hazard ratio, 2.22; 95% CI, 1.37 to 3.58; $P = 0.001$) and endothelial donor-specific antibody-selective transcripts (hazard ratio, 3.02; 95% CI, 1.00 to 9.16; $P < 0.05$) were independently associated with an increased risk of graft loss. Moreover, adding this gene expression assessment to a traditional risk model improved the stratification of patients at risk for graft failure.[55] Molecular microscopes might also help physicians to assess the response to rejection treatment and may help to adjust the immunosuppressive therapy.

Our new understanding of the nature and pathogenesis of DSAs and how they relate to kidney biology will inevitably lead to changes and improvements in treatment approaches for kidney transplant patients. One suggestion would be to improve therapeutic outcomes by focusing on AMR prevention rather than treatment after its appearance.[56] However, to achieve this, effective screening procedures and improved kidney allocation policies must be in place. Other suggestions to improve treatment outcomes include avoidance of transplantation in patients with preexisting DSAs, screening and active management of nonadherence to immunosuppressive treatment, and reinforcement of adequate exposure to tacrolimus both early posttransplant and over the long term. By modifying these risk factors with the immunosuppressive regimens administrated to our patients we aim to reduce the occurrence of DSAs and improve patients' outcomes. Given the involvement of distinct subclasses of DSAs in kidney allograft injury, these characteristics should also be taken into account beyond simply testing patients positive for DSAs; an unmet need is to recognize that the population of preformed and recurrent DSAs is different from the de novo DSA population. As we move toward a more personalized approach to kidney transplantation and redefine what "best practice" treatment looks like, our ability to integrate all available and multidimensional data, including phenotypic, histopathologic, transcriptomic and immunologic information, will prove critical to identify appropriate treatment approaches for different patients.

Nonadherence to Treatment in Kidney Transplant Recipients

Prof Christophe Mariat

Nonadherence to immunosuppressive treatment in kidney transplant recipients is well established as a risk factor for poorer long-term outcomes; however, identification of nonadherent patients before the onset of clinical consequences can be difficult.[57-59] In 2009, The American Society of Transplantation defined nonadherence as a deviation from the prescribed medication regimen that is sufficient to adversely influence the regimen's intended effect.[60] Many factors have been associated with nonadherence in the literature and some of those factors, such as age, social factors, education, type of employment, history of nonadherence with a previous transplant, and history of addiction, are related to patient characteristics. Other factors include those that are directly related to the treatment: the taste and the size of the pill, anticipated or experienced adverse events, and the complexity of the drug regimen.[61] Siegal and Greenstein classified 3 different types of nonadherence profiles based on patient behavior/belief.[62]

1. Accidental: patients who forget to take their medication (47% of nonadherent patients fall into this category)

2. Invulnerable: patients who feel that they do not need to take their medication (28%)
3. Decisive: patients who actively decide not to take their medication based on an observed or scientific rationale (25%)

Before an assessment of the prevalence of nonadherence in transplantation is attempted, it is very important to stress the difficulty of accurately measuring adherence to treatment. The "gold standard" would be to actually witness the drug being taken by the patient, but obviously it is very difficult to implement this method in practice. Therefore, it is necessary to become familiar with drug-monitoring systems. Indirect methods of measuring adherence to treatment include pill counts, pharmacists' refill records, self and collateral reports, and interviews. Electronic monitoring devices are usually considered the "reference" for measuring nonadherence in clinical research, although no actual recognized gold standard currently exists. For this reason, to make an accurate assessment of the prevalence of nonadherence in our patients, it is necessary to combine different methods of data collection.

Schäfer-Keller et al[59] reported a 12% to 39% nonadherence rate in the population of kidney transplant recipients studied, demonstrating that the prevalence of nonadherence was dependent on the measurement used. None of the methods they used in their study are considered good independent diagnostic measures of adherence. The highest specificity was achieved by the interview technique, but only when the interview had been conducted by 3 or more different physicians, and the highest sensitivity was achieved with the composite score. These data indicate that a well-defined composite score for nonadherence could be a beneficial tool in clinical practice. Not surprisingly, therefore, the incidence of nonadherence in kidney transplantation reported in the literature is variable. In a study by Dharancy et al,[63] the prevalence of nonadherence measured by physician assessment was reported as 47% for kidney transplant patients versus 49% for liver transplant patients. When nonadherence was reported using a patient self-assessment method, there was a significant difference in the incidence reported. In 61.6% of cases, physicians considered their patients to be adherent to treatment, whereas the patients considered themselves to be only moderately or poorly adherent.

Nonadherence to treatment has been shown to increase over time and different types of nonadherence can have different impacts on clinical outcomes. In a French cohort, 35% of patients were nonadherent to treatment by year 2 posttransplant.[64] When the nature of the nonadherence was assessed, it was thought to be partially as a result of not taking the medication at the appropriate time, which may not be as dangerous as not taking the medication at all.

The clinical consequences of nonadherence to treatment are widely documented in the literature, and there is emerging evidence to suggest that nonadherence is a risk factor for late AR and graft loss.[57,58] In 2012, Sellarés et al[57] outlined what is now considered to be the natural history of graft failure, highlighting that there is a direct link between the development of DSAs, nonadherence to treatment and graft loss. Of the patients in this study who experienced graft failure, 47% were considered to be nonadherent to treatment (Figure 6).

To summarize, nonadherence to treatment in transplantation is well defined; however, the occurrence and type of nonadherence is not easy to capture, and there is a need to use

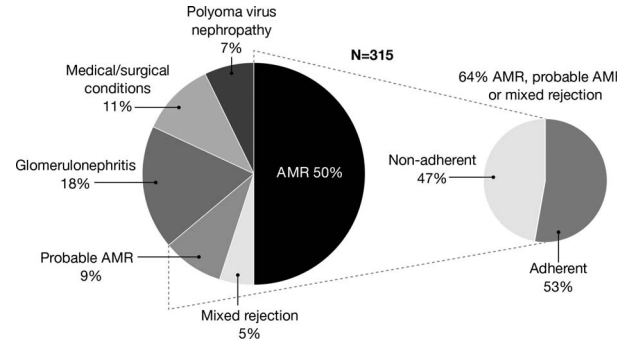


FIGURE 6. Distribution of the attributed causes of graft failure in the biopsy-for-cause population. Failures that could not be attributed, owing to missing clinical information, are not represented (n = 4). AMR, antibody-mediated rejection. Reprinted with permission from Sellarés J, de Freitas DG, Mengel M, et al. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. *Am J Transplant.* 2012;12:388–399.

different methods of diagnosis to get an overall picture that is truly reflective of solid organ transplantation. A number of new technologies aiming to address the issue of nonadherence are available, including interactive voice response systems, ingestible sensors and smartphone apps. Nonadherence is associated with high clinical and financial burden, rejection, graft loss, return to dialysis, and retransplantation. We should systematically and longitudinally assess all our patients' adherence to their medication, not only those who are presumed to be at high risk of being nonadherent. Taking this approach to managing nonadherence and introducing innovative technologies to support patients to modify their behavior will make steps towards improved outcomes over the long term.

Prof Dirk Kuypers

To improve our patients' outcomes we must manage nonadherence to treatment; however, this management, in any therapeutic setting, has to be accompanied by techniques to identify nonadherence in the patient population. Two systematic reviews on nonadherence to treatment, encompassing approximately 37 studies, assessed interventions in different therapeutic areas. For patients who received education alone, the improvement in adherence was small. When a behavioral intervention was implemented (reduction in the burden of tablets), then the effect size was larger. When both strategies were implemented together, the magnitude of the effect was further increased; this was dependent on how many times the patients were reeducated about the risks of nonadherence to treatment.[65,66]

In transplantation, there are also 2 strategies to manage nonadherence; either the implementation of educational patient programs or simplifying the dosing regimen of the medication. When prolonged-release tacrolimus (Advagraf, tacrolimus prolonged-release hard capsules) came to the market, we decided to determine if using a simplified regimen with this drug really improved adherence to treatment. The ADMIRAD study (ADherence Measurement In stable Renal transplant patients following conversion from Prograf (tacrolimus hard capsules) to ADvagraf) assessed adherence to the immediate-release tacrolimus formulation for 3 months, then patients were randomized to continue treatment or to convert (1:2 ratio) to the prolonged-release formulation and monitored for a further 6 months. Adherence was measured by a

Helping Hand device (Bang & Olufsen Medicom, Denmark); the used blister package is inserted into the electronic device after taking a tablet and the date and time is registered. Interestingly, in this study, patients were less adherent to the afternoon versus morning dose of immediate-release tacrolimus.[67] This was thought to be related to less stringent routines in the afternoon.

For the implementation indicator, that is, percentage of medication taken, on a day-to-day basis, a significant improvement in adherence of 10% was achieved with prolonged-release versus immediate-release tacrolimus (Figure 7).[67] When the time of administration was included in the analyses, to give a more stringent assessment of adherence (±2 hours), a significant difference of 10% was still observed. However, the 10% difference between the 2 groups was not statistically significant when persistence was measured, that is, patients who remained on the prescribed dosing scheme.[67]

The Helping Hand device was able to detect different patterns of nonadherence in our patients. We observed patients who were occasionally nonadherent to their treatment, those who took their doses later than prescribed during the weekends, those who were more likely to miss the evening versus the morning dose of immediate-release tacrolimus and those who became increasingly nonadherent over time.[67]

The Medication Adherence Enhancing Strategies in Transplantation study group are conducting a study in solid organ transplantation (heart, liver, lung), examining adherence in patients on a twice-daily tacrolimus-based regimen who were randomized to one of 2 arms: Arm 1 included patients who received an educational refreshment course, feedback on the printout of their Helping Hand devices, motivational interviews, reminder systems and training in problem-solving skills; Arm 2 included patients who were given the readouts from their Helping Hand device at each study visit. This study is useful as it assessed the combined effect of motivational and educational support. These kind of new study initiatives have encouraged us to expand our trials to look at patient lifestyle and general health issues in an attempt to further improve adherence to treatment.

With this in mind, we are in the process of setting up a new study in our clinic. After interviewing a sample of our patients we have decided not to use a smartphone app for the trial, as only a minority have suitable devices. Our patients also told us that entering data into a computer each day would be a barrier to the trial, as it would interfere too much with their regular daily routine. Therefore, we are taking a new approach; we have provided a platform where the patient does not have to input their own data. Adherence to treatment will be measured by a pill bottle that is electronically monitored, and physical activity and weight will be monitored by a step meter and scales, respectively, which automatically record the information. We expect to have results from the Picasso-Tx Study (is there a Preference for InteraCtive Health Technology Applications to support Self-management in Solid Organ transplant (Tx) recipients?) in 1 to 2 years.[68]

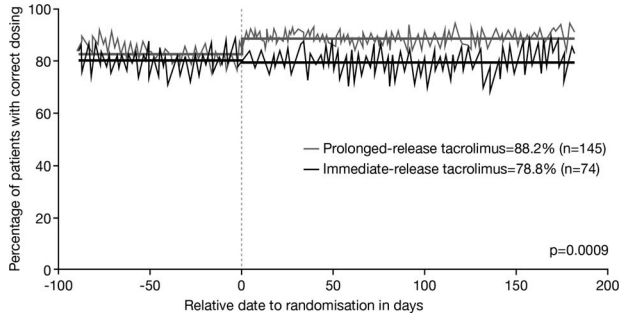


FIGURE 7. Implementation of dosing regimen with prolonged-release versus immediate-release tacrolimus. Correct dosing is defined when the medication intake that day is at least as prescribed. Dashed line at Time 0 represents time of randomization. The overlaying lines are model-based estimations of the day-to-day percentages. Reprinted with permission from Kuypers DR, Peeters PC, Sennesael JJ, et al. Improved adherence to tacrolimus once-daily formulation in renal recipients: a randomized controlled trial using electronic monitoring. *Transplantation*. 2013;95: 333–340.

Finally, I want to discuss how you can intervene in a clinical setting to improve patient adherence to treatment. This is a proposition that we have, at least in part, implemented in our hospital. During pretransplant consultations, patients have the opportunity to discuss their care and the importance of adherence to treatment with a multidisciplinary team, including transplant nurses/nurse specialists, the attending physician, the transplant coordinator, a dietician and psychologist(s), and so on. On day 5 posttransplant, we conduct routine monitoring and initiate an educational program with the patients. At month 3, when we take a protocol biopsy, we implement an educational refresher course and a question and answer session about lifestyle, medication intake, side effects, diet, and a number of other factors with the responsible nurse. Variability of tacrolimus exposure is also a potential flag for nonadherence, and this is discussed with the patient. At month 12, we repeat the interventions if a patient has requested information involving their medication regimen or lifestyle. Perhaps implementing this type of program over a longer period would be beneficial in improving medication adherence and, subsequently, long-term outcomes. However, we would need to consider the technology and practicalities that can be used for these interventions in clinical practice. In addition to the technologies already discussed, dry blood spot monitoring to allow tacrolimus trough levels to be measured at home, nonadherence questionnaires, such as the Basel Assessment of Adherence with Immunosuppressive Medication Scale (BAASIS) questionnaire, and different smartphone apps, like the risk factor calculator (Astellas Pharma Europe Ltd, UK), could be of assistance.

In conclusion, nonadherence to treatment in transplantation is more frequent than we initially suspected and can be determined and monitored using a combination of instrumental tools and targeted questioning of patients. Nonadherence to treatment is a risk factor for poorer outcomes in solid organ transplantation, including late AR, the development of DSAs, chronic graft injury and graft loss.[57] Treating nonadherence in clinical practice is actionable and manageable and can make a significant difference to the long-term outcomes of our patients.[67] Reducing the pill burden and implementing/repeating educational and support programs have the potential to achieve significant and long-lasting improvements for the outcomes of our patients, and we eagerly await data from ongoing studies in our clinic.

Variability of Immunosuppressive Exposure in Kidney Transplantation

Mr Marc Clancy

Historically, reducing the incidence of AR has been the main focus in clinical practice. However, new advances in immunosuppressive regimens have led to a shift in focus

from short-term to long-term outcomes. The Symphony study provided evidence for improved 1-year outcomes with tacrolimus plus mycophenolate mofetil (MMF) and corticosteroids compared with the other regimens studied.[69] This regimen has been applied in an unselected "real world" population of patients in our unit, and has provided benefits in terms of reducing rejection and graft failure, and improving renal function in the first year posttransplant.[70] However, longer-term success requires modification of the key risk factors associated with poor graft and patient survival. Many of these are generic to all individuals, such as diet and lifestyle, but the key modifiable factor for a transplant patient is optimization of maintenance immunosuppression.

Because tacrolimus is a narrow therapeutic index drug, small variations in systemic exposure can lead to large differences in the pharmacodynamic response. Overimmunosuppression is associated with an increased incidence of infectious, toxic and malignant adverse events, while underimmunosuppression can lead to a breakthrough of the alloimmune response manifesting as AR, AMR, and/or the emergence of de novo DSA.

Immunosuppression exposure is usually measured via the surrogate, trough tacrolimus level. Variability of tacrolimus exposure occurs both between individuals, and within the same individual at different time points. For this reason, both interpatient and intrapatient variabilities must be taken into consideration when managing patients in clinical practice. Many of the factors associated with interpatient variability (sex, *CYP3A5* polymorphisms, and so on) are constant and their effects occur early, allowing active clinical management to resolve any potential problems. In contrast, the modifiable risk factors that affect intrapatient variability are ongoing and relate, in part, to adherence behaviors: taking the drug at the right time with the right stomach conditions (food/no food) and other lifestyle factors that affect trough levels, as well as the practical factors associated with measuring trough levels.

Intrapatient variability of tacrolimus exposure is typically measured by the percentage deviation from the mean level of all trough levels within a given time period or by the coefficient of variation of the same set of levels. It is widely accepted that graft and patient survival can be affected by large falls or rises in tacrolimus trough levels, well outside the target range; however, the clinical impact on the patient of small fluctuations outside the therapeutic range also appears to be important. As early as 2000, Kahan et al[71] demonstrated that patients in a higher-variability cyclosporine cohort exhibited a higher incidence of graft loss compared with patients in the lower-variability cohort. Similar findings regarding the impact of cyclosporine variability on long-term outcomes were also reported by Waiser et al.[72] In a study of 46 pediatric transplant recipients, higher variability of tacrolimus exposure was associated with the presence of acute rejection,[30] and Borra et al[31] reported a significant relationship between high within-patient variability of tacrolimus exposure and long-term graft failure. Furthermore, in a recent study of 220 kidney transplant recipients, higher tacrolimus intrapatient variability predicted accelerated progression of chronic histologic lesions before onset of renal dysfunction.[73]

The lower-dose tacrolimus plus MMF and steroid regimen used in the Symphony study is the most commonly used in clinical practice. In a subanalysis of data from this study, intrapatient variability of tacrolimus exposure of ~28% was apparent with this regimen in the first 12 months. Of interest is that only 11% of patients had trough levels that were within the target range at all times during the first 2 months posttransplant.[74] Armed with the information that a higher variability of tacrolimus exposure leads to poorer long-term outcomes,[31] best practice should now be to recognize high variability of tacrolimus exposure as a risk factor for poor long-term outcomes, and to modify this risk factor in clinical practice. By making changes to the immunosuppressive regimen and reducing the variability of tacrolimus exposure, further improvements in long-term outcomes for kidney transplant recipients may be achievable.

When we assessed the variability of tacrolimus exposure in our clinic, we did not expect high variability to be identified in our patients. However, data from 255 prospectively compiled patient records 1 year posttransplant confirmed significantly higher rates of AR and graft loss for patients with variability above the median of all levels between 6 and 12 months posttransplantation, compared with patients with variability below or equal to the median.[75] In the first 6 months posttransplant, exogenous treatments with steroids and antibiotics to treat graft failure, AR and infection are frequently applied, and could affect tacrolimus levels by artificially raising the variability of exposure. Further investigations of data from 376 patients over a 4-year period confirmed the divergence in graft survival between the higher-variability versus lower-variability cohorts in our clinic, amounting to an ~12% difference. Significant renal function benefits were also observed in the lower- versus higher-variability cohort (n = 326; rejection-free patients only). Analysis of DSA measurements demonstrated that DSA-negative patients had significantly lower tacrolimus variability profiles compared with DSA-positive patients (n = 235; 16% versus 25%, respectively; Figure 8 [M. Clancy, written communication, January 25, 2015]). Therefore, a possible explanation for the link between higher variability of tacrolimus exposure and formation of DSAs could be that fluctuations in the immunosuppressive regimen allow a 'break-through' of the immune system.

With any data set or analysis it is important to recognize the limitations when interpreting and applying the findings to clinical practice. The data on variability of tacrolimus exposure that are currently available have generally been generated via retrospective analysis and covariation with factors, particularly nonadherence, and are impossible to

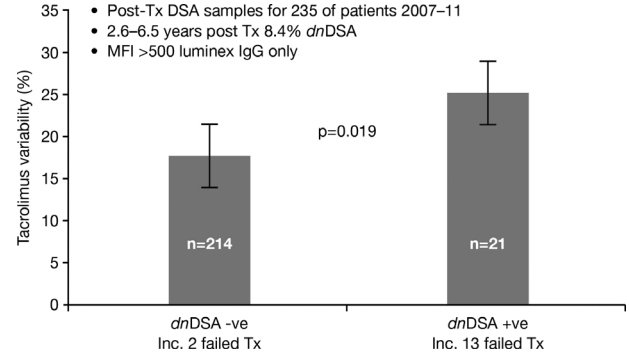


FIGURE 8. Potential link between variability of tacrolimus exposure and de novo DSA in kidney transplantation (M. Clancy, written communication, January 25, 2015). DSA, donor-specific antibody; dnDSA, de novo donor-specific antibody; IgG, immunoglobulin G; MFI, mean fluorescence intensity; Tx, transplant.

fully dissect. The ADMIRAD study demonstrated that adherence to tacrolimus improved by 10% when patients were converted from immediate-release to prolonged-release tacrolimus.[67] Converting patients from immediate-release to prolonged-release tacrolimus preparations has been shown to reduce intrapatient variability in both South Asian[76] and European populations.[77] The clinical consequences of this degree of improved adherence cannot be easily mapped, but logically, a high-variability cohort should benefit in terms of clinical outcome measures from a once-daily, prolonged-release formulation of tacrolimus.

Prospective interventions to reduce intrapatient variability start with the identification of patients with a high variability of tacrolimus exposure. This can be achieved via a manual calculation using a spreadsheet or via an app, such as the variability calculator (Astellas Pharma Europe Ltd, UK), which calculates variability (coefficient of variation) from tacrolimus trough measurements. Once patients with high variability have been identified (according to the preset parameters in the app or based on the clinic's own cohort of patients), interventions could be beneficial. These interventions include education on the impact of variability and nonadherence to treatment, and conversion to prolonged-release tacrolimus, which has been shown to have lower variability of exposure and to improve adherence to treatment versus immediate-release tacrolimus.[67,77]

High intrapatient variability of tacrolimus exposure defines a group of patients proven to manifest rejection, graft failure and dysfunction at a higher rate than the lower-variability population. Intervention to reduce variability would, therefore, seem justified and sensible. More evidence to establish the magnitude of improvement in long-term outcomes, due to the lower variability in tacrolimus exposure with the prolonged-release versus immediate-release formulation is warranted. However, based on the information currently available, conversion to a once-daily tacrolimus preparation alongside additional supportive measures is a logical approach to reducing intrapatient variability.

Underimmunosuppression in Kidney Transplantation

Dr Luís Guirado

Tacrolimus remains the cornerstone of immunosuppressive treatments, with tacrolimus plus MMF and steroids being the most commonly used regimen in kidney transplantation; however, our understanding of immunosuppressive regimens is evolving. Ten years ago, nephrotoxicity was considered to be a major risk factor and tacrolimus minimization was readily discussed in an attempt to prevent kidney damage and improve outcomes for our patients. We now understand that the histologic lesions classically attributed to nephrotoxicity are nonspecific and the main cause of these lesions is alloimmunity.

In order to avoid nephrotoxicity, there is a temptation to overminimize tacrolimus trough levels. Five-year posttransplant graft survival data reported by the CTS show that if patients are maintained on trough levels less than 5 ng/mL at year 1 posttransplant compared with trough levels of 5 to 7 ng/mL, 7 to 10 ng/mL and greater than 10 ng/mL, they are at higher risk of graft failure (Figure 4).[25] Patients with 1-year creatinine levels 130 to 250 μmol/L show improved renal function 5 years posttransplant; however, patients with 1-year serum creatinine levels less than 130 μmol/L were found to have similar serum creatinine levels 5 years posttransplant regardless of tacrolimus trough levels. The 5-year findings reported by the CTS for patients receiving tacrolimus plus MPA with serum creatinine levels less than 130 μmol/L at 1 year demonstrate that maintaining tacrolimus 5 ng/mL or greater versus less than 5 ng/mL has a renal function benefit over 5 years of treatment.[25] CNI avoidance strategies, CsA in this example, have also been associated with earlier and more frequent de novo DSAs and an increased incidence of AR compared with standard-dose regimens.[38] Assessment of the development of DSAs should also be checked at least once per year.

In our experience, patients who had inflammation in their protocol biopsy had significantly lower tacrolimus trough levels ($P \leq 0.04$)[78] and higher blood creatinine levels ($P = 0.003$) versus those without inflammation in their protocol biopsy. There was a more pronounced difference in the high immunologic risk patients ($P < 0.001$). A higher proportion of the patients in the deceased donor group compared with the living donor group had inflammation at 12 months posttransplant. We hypothesize that this is due to the lower tacrolimus trough levels (5-6 ng/mL) in this group and that 7 to 8 ng/mL of tacrolimus exposure should be targeted after 1 year posttransplant.

The question remains, how can we minimize CNI nephrotoxicity and preserve renal function without compromising immunosuppression? We have established that maintaining tacrolimus trough levels of 5 ng/mL or greater is crucial in kidney transplantation. It is also essential to ensure consistent tacrolimus exposure over time, as oscillating tacrolimus levels could subject patients to overexposure of tacrolimus, with the risk of toxicity, or underexposure, with the risk of rejection. We found in our clinic that patients receiving prolonged-release tacrolimus had lower intrapatient variability of exposure versus patients receiving the immediate-release formulation. This could have been, in part, due to improved adherence. In the EVOLUTION study (EValuation Of Advagraf conversion and Long-term Use in kidney TransplantatION), for example, a 34% improvement in adherence to tacrolimus was observed in the patients receiving the prolonged-release versus immediate-release formulation.[79] These findings were consistent with Stifft et al[77] who reported a 23% reduction in intrapatient variability after conversion from immediate- to prolonged-release tacrolimus. Previously published studies have also demonstrated a lower variability of tacrolimus exposure and improved adherence to treatment with prolonged-release versus immediate-release tacrolimus.[76,77,80]

Previously reported data from 40 000 patients over 6 years of follow-up suggest that there is an annual decrease in renal function in the posttransplant period of ~1.6 mL/min per 1.73 m^2 per year,[81] with similar findings being reported for 4000 kidney transplant patients in the Catalan Registry (–1.5 mL/min per 1.73 m^2 per year). These data suggest that even 'stable' patients are experiencing a decline in renal function over time and that we need to continue to monitor this decline and to modify our therapeutic regimens to improve outcomes for our patients. A number of recent publications have found prolonged-release tacrolimus to be associated with a reduction in renal function impairment compared with the immediate-release formulation (Figure 9).[82-84] We speculate that this could be due to a more consistent pharmacokinetic profile of prolonged-release versus immediate-release tacrolimus.[85]

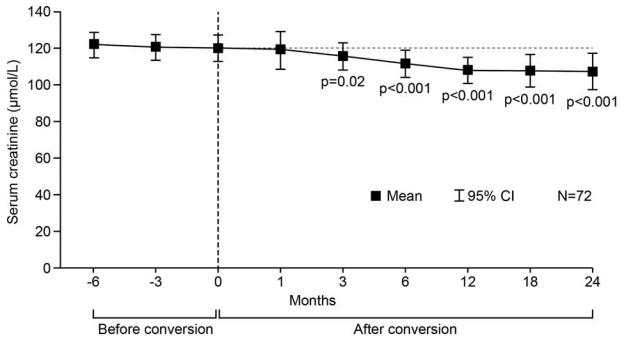


FIGURE 9. Mean serum creatinine concentration before and after conversion from immediate-release to prolonged-release tacrolimus in kidney or pancreas–kidney transplant patients. *P* values calculated using the Student t-test. CI, confidence interval. Figure adapted with permission from Kolonko A, Chudek J, Wiecek A. Improved kidney graft function after conversion from twice daily tacrolimus to a once daily prolonged-release formulation. *Transplant Proc*. 2011;43:2950–2953 doi:10.1016/j.transproceed.2011.07.014. Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

Data from 1832 patients converted from immediate-release to prolonged-release tacrolimus in the EVOLUTION study[79] showed that tacrolimus trough levels decreased by 10% immediately after conversion; however, postconversion dose and trough levels remained stable over 12 months of treatment. In these patients, renal function was well preserved, with a decrease lower than that of the general transplant population over the first year (–0.76 mL/min per 1.73 m^2).[79] AR was low (0.4%) and 99.4% of patients reported a positive impression postconversion.[79] In the follow-up study, REVOLUTION (n = 1798),[84] tacrolimus dose and trough levels were maintained over a further 3 years of treatment. A significant reduction in the intrapatient variability of tacrolimus trough levels was reported at year 4 compared with month 0 (preconversion) (P = 0.01). Renal function decline remained low compared with the general transplant population throughout the follow-up.[81,84] The difference between the expected and the observed annual decrease in renal function over 4 years is both significant and clinically relevant to our patients. The best practice care for our patients is to monitor their renal function and to perform a protocol biopsy at 1 year posttransplant. If patients do not have proteinuria but have some degree of inflammation, it is likely that fibrosis will occur in the future and we should take steps to modify the risk factors associated with underimmunosuppression, including improving adherence to treatment and minimizing the variability of tacrolimus exposure. We are currently working to identify biomarkers to identify patients with inflammation, as inflammation is the principal cause of graft loss.

To conclude, tacrolimus plus MMF and steroids is the most used combination of immunosuppressive drugs in kidney transplantation. It is now accepted that nephrotoxicity is much less frequent than first considered, and although there is a general consensus that tacrolimus trough levels must be maintained 5 ng/mL or greater, we need to move forward by defining the optimal tacrolimus trough levels for our patients in both the early transplant period and during maintenance therapy. Tacrolimus avoidance or overminimization can increase the development of DSA and humoral rejection, and lead to poor graft survival. Prolonged-release tacrolimus helps to maintain renal function[79,82-84]; this could be due to improved adherence to treatment and decreased intrapatient variability, and by maintenance of an adequate balance between overimmunosuppression and underimmunosuppression.

Cardiovascular Complications in Kidney Transplantation

Prof Bengt Fellström

While life expectancy posttransplant is improving in the kidney transplant population, the incidence of cardiovascular events remains largely constant; approximately 40% of deaths with a functioning graft can be attributed to cardiovascular disease in the first year posttransplant.[86] Patients with chronic kidney disease have an increased risk of cardiovascular disease that increases with progressive renal dysfunction and peaks during dialysis. Although the risk is lower posttransplant, patients still have a higher risk of premature cardiovascular disease compared with the general population.[87]

Risk factors for cardiovascular complications can be divided into factors that exist before transplantation and factors that develop posttransplant. Pretransplant risk factors include age, sex, previous cardiac and vascular disease, total time on renal replacement therapy, smoking status, and hyperlipidemia. Posttransplant risk factors include left ventricular hypertrophy, hypertension, renal transplant dysfunction, NODAT and hyperlipidemia. Some of these risk factors can be modified by the immunosuppressive regimen and treatments that are administered posttransplant.

Data from the United States Renal Data System database show a prevalence of 42% of NODAT in kidney transplant recipients.[88] NODAT is associated with increased cardiovascular risk and has a negative impact on graft function.[89] Both pretransplant diabetes and NODAT are associated with significantly poorer patient survival compared with absence of diabetes.[90] A number of immunosuppressive therapies, including corticosteroids, tacrolimus, CsA and mammalian target of rapamycin (mTOR) inhibitors, have been associated with a higher risk of developing NODAT.[91] The role of steroids in the development of NODAT has been well established; data from an analysis of greater than 25 000 kidney transplant recipients demonstrated that patients who were receiving steroids at the time of discharge from hospital had a 42% greater risk of developing NODAT.[92] The management of patients with NODAT follows the same stepwise approach applied to the general population, including lifestyle and dietary modifications, pharmacologic therapy and patient monitoring.

Renal allograft decline and subsequent dysfunction are associated with an increased risk of cardiovascular events. Fellström et al[93] reported that elevated serum creatinine at baseline was a strong and independent risk factor for major adverse cardiac events (MACEs), and cardiac and all-cause mortality. A correlation between graft loss and nonfatal myocardial infarction was also reported. In a separate study of 1120295 adults (nontransplant), a decline in renal function was associated with an increased risk of the occurrence of cardiovascular events.[94] Glomerular filtration rates (GFRs), estimated by the modification of diet in renal disease (MDRD) formula, of 15 to 29 and less than 15 mL/min per 1.73 m^2 in the absence of dialysis, were associated with a higher incidence of age-adjusted mortality.[94]

Hyperlipidemia has also been studied for cardiovascular complication in kidney transplantation. In the ALERT

(Assessment of LEscol in Renal Transplantation) trial, low-density lipoprotein cholesterol was associated with an increased incidence of nonfatal myocardial infarction.[95] Long-term benefits of statin therapy were demonstrated in the trial, leading to changes in best practice and the implementation of early treatment for patients with high cholesterol to be included in guidelines for the management of renal transplant recipients.[96]

The influence of immunosuppressive drugs on cardiovascular risk factors has never been systematically estimated, although from a semiquantitative evaluation it has been suggested that corticosteroids, tacrolimus and CsA influence hypertension, hyperlipidemia and NODAT, whereas drugs like MMF and monoclonal antibodies seem to be quite neutral with regard to cardiovascular risk.[87] Inflammation may have an impact on cardiovascular risk in kidney patients. Data from the ALERT trial demonstrated that biomarkers such as interleukin-6 and C-reactive protein have a direct association with increased cardiovascular risk.[97]

The Framingham risk factor model has not been validated in kidney transplantation and, owing to the multifactorial specificity of kidney transplant patients, it is not considered to be applicable for calculating cardiovascular risk in these populations. Therefore, there is an ongoing need for the development of a risk factor calculator that is specific to kidney transplantation. The MACEs risk calculator that we developed comprises a 7-variable model (age, previous coronary heart disease, diabetes, low-density lipoprotein, creatinine, number of transplants, and smoking status), and the mortality risk calculator comprises a 6-variable model (age, previous coronary heart disease, diabetes, creatinine, total time in renal replacement therapy, and smoking status).[98] These risk factor models were calibrated using the Hosmer–Lemeshow test, applied to a data sample from the ALERT trial, and validated using data from the PORT study (Patient Outcomes in Renal Transplantation).[98]

These risk factor equations were applied to the BENEFIT (Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression Trial) and BENEFIT-EXT studies (BENEFIT-EXTended criteria donors), to estimate MACE and mortality in patients enrolled in the belatacept LI (less intensive) and CsA arms.[99] Over 7 years, the model predicted that patients treated with belatacept would result in a significant reduction in mortality in BENEFIT (5% absolute reduction) and BENEFIT-EXT (5.64% absolute reduction) versus CsA. As expected, owing to the use of ECDs in the BENEFIT-EXT study, a lower incidence of MACE was predicted in the BENEFIT (3.40% absolute reduction) versus the BENEFIT-EXT trial (4.88% absolute reduction).

Risk factor equations, with appropriate validation, may provide us with useful insights into the long-term potential of different treatment regimens in the absence of long-term clinical trials. Taken in context with clinical trials and real-world data, including registry data, these calculations could help us to better determine the best practice care in kidney transplantation. An adequately validated risk predictor may also be used for individual counselling of kidney transplant patients in an attempt to reduce cardiovascular risk, but this application requires clinical judgment and depends on the context for each patient.

In conclusion, renal transplant recipients have an increased risk of cardiovascular events, but the risk is lower than for patients who remain on dialysis. Risk associated with cardiovascular disease is complex to predict and each factor needs to take into account a number of different parameters, including age and gender. Additional research is needed to further explore the specific risk factors for posttransplantation cardiovascular disease, and recommendations for best-practice management and treatment of these are still required. In the meantime, some of the cardiovascular risk factors are modifiable, and by choosing the optimal treatment regimens we can improve the long-term outcomes for our patients.

The Liver Graft Journey and Risk Management

Prof John O'Grady

Liver transplantation has the ability to restore many decades of health to patients with a range of life-threatening conditions. The concept of the graft's journey captures the challenges facing the organ if it is to deliver its full potential. In this overview, the journey is divided into 7 components, ranging from the point of implantation to the time the organ ceases to function, ideally after taking the recipient to an age that fulfils normal life expectancy for that individual. A number of the issues are considered in more depth in the chapters that follow.

Implantation

Primary nonfunction and poor early dysfunction represent suboptimal starts to the graft journey. Primary nonfunction appears to be occurring less frequently than in previous years, but when it occurs, the management remains immediate retransplantation. There are numerous associations with poor early graft function, including severe steatosis, prolonged cold ischemia time and donation after cardiac death. Extracorporeal machine perfusion, including normothermic and hypothermic techniques, is beginning to show promise in improving early graft function and is potentially a major advance for the future.

Establish Immunologic Stability

The initial immunosuppression regimen aims to achieve immunologic stability of the graft. A CNI-based approach using lower doses than historically practiced is now broadly accepted as standard. Biologic agents are sometimes used as routine therapy, but more frequently as an adjunct to reduced-dose or delayed exposure to CNIs. One or 2 additional drugs are typically included in the initial regimen. There is increasing acknowledgement that the rate of acute cellular rejection (ACR) in the first 10 days is not an appropriate metric of the efficacy of the initiating immunosuppression strategy.

Maintain Immunologic Stability

It has been stated that protocol immunosuppression is largely an outdated concept.[100] Instead, maintenance immunosuppression is personalized to reflect the needs of individual patients, balancing good graft function with the lowest achievable profile of side effects attributable to the totality of the immunosuppression as well as the individual agents being used. It is also en vogue at present to minimize the dose of the CNIs, a practice that has, to a large degree, been driven by a desire to protect renal function over the long term. There was no evidence that this approach increased the risk of chronic rejection and the incidence of ACR was only increased when CNIs were avoided.[101] There are theoretical concerns that unintended consequences of minimization of

immunosuppression might be permissive for graft injury through AMR, plasma cell hepatitis and idiopathic fibrosis.

Address Technical Complications

Of all the elements of the journey, this is the one that is currently the least active in delivering improved outcomes. Stricture formation at the site of the biliary anastomosis is the most frequently encountered technical complication. Detection is typically by magnetic resonance cholangiography, which is followed by evaluation of clinical significance using direct cholangiography and possibly liver biopsy, particularly if surgical reconstruction is being considered. Primary management is usually balloon dilatation and stent placement, with surgery being reserved for persistent stricturing. Diffuse cholangiopathy complicates microvascular and macrovascular injury or recurrence of primary sclerosing cholangitis. Management is challenging and more severe cases require retransplantation.

Vascular complications may involve any of the anastomosed vessels. The most significant is hepatic artery thrombosis and the earlier it occurs after liver transplantation, the more likely the need for retransplantation. Graft infarction, liver abscesses, and ischemic cholangiopathy represent 3, usually discrete, manifestations of arterial thrombosis. Venous outflow complications are more likely with caval preservation techniques.

Prevention of Recurrent Disease

This has been the most significant of the challenges to the functioning graft. However, there is a strong expectation that HCV, which was the most challenging of the recurring diseases, will be effectively managed with the emerging direct-acting antiviral agents.[102,103] Affordability rather than efficacy is the immediate concern in this regard. Alcohol-related and non-alcohol-related fatty liver disease and the autoimmune diseases are the next most important of the diseases with the potential to recur. Within these aetiologies there is considerable variation in the risk and clinical consequence of the recurrent disease; HCC recurs in about 15% of patients, despite careful selection, and is rarely amenable to therapy.

Management of Drug-Related Toxicity

In addition to the generic burden of immunosuppression, there is a range of toxicity profiles that have the potential to impact on the graft journey. Historically, CNI-related nephrotoxicity has been the exemplar of this consideration because of apparent causative association, coupled with potentially serious outcomes. A sophisticated response strategy has emerged that starts in the immediate posttransplant period and runs throughout the journey of the graft. It begins with delayed or reduced exposure to the CNI and continues with individualized dosing regimens that give priority to the long-term maintenance of good renal function. The latter includes attention to other processes that contribute to loss of renal function, with hypertension and diabetes mellitus being leading examples (drug toxicity contributes independently to both). The risk of cardiovascular disease is another dimension of these side effects, in addition to dyslipidemia and obesity. The risk-management strategy for cardiovascular disease is less well defined but is likely to be modifiable with intelligent utilization of the therapeutic options for effective immunosuppression.

Death With a Functioning Graft

The ultimate hope for a transplanted organ is that it helps sustain the life and health of the recipient until at least normal life expectancy is realized. This has been achieved many times for individuals, but it is only recently that evidence has emerged to indicate that it is becoming a realistic ambition for some patient cohorts. A single-center, 20-year follow-up study indicated that patients over 55 years of age who were alive 1 year after liver transplantation had life expectancies comparable to the normal population.[104] At present, the greatest shortfall in life expectancy in the first 20 years is in those aged 30 to 55 years at the time of transplantation. The 4 main causes of premature mortality are recurrent disease, infection, malignant disease and cardiovascular disease. The expectation for the future is that each of these will gradually reduce in importance until the aspiration of delivering normal life expectancy is routinely realized.

Donor-specific Alloantibodies in Liver Transplantation

Dr Jacqueline G O'Leary

The liver is relatively resistant to acute AMR compared with other solid organs transplanted.[105,106] This resilience is facilitated by numerous mechanisms, but several are of critical importance. The liver's size, affording it a 100-fold microvasculature compared to the kidney, regenerative capacity, ability to secrete soluble class I antigen, and the existence of hypocomplementemia (secondary to liver dysfunction) serve to mollify the effects of preformed DSAs. Furthermore, even in the presence of injury, immune complexes and activated complement are phagocytized by Kupffer cells. However, in cases when acute AMR does occur in liver transplantation, it is associated with poorer clinical outcomes.[4]

Despite the rarity of acute AMR, it can now be definitively diagnosed. Key histopathologic characteristics include portal vein endothelial cell hypertrophy, portal eosinophilia and eosinophilic venulitis (Figure 10).[106] The presence of lymphocytic portal inflammation and lymphocytic venulitis without the aforementioned features favours cellular rejection as opposed to combined cellular- and AMR; acute AMR is almost never found in isolation. In 1 retrospective, multicenter study, evaluation for these key features using the acute AMR score allowed for acute AMR determination with 81% sensitivity and 71% specificity.[106] However, when acute AMR is suspected, one must also test for DSA, stain for C4d, and rule out other causes of a similar injury pattern.[107] Clinical features, such as falling platelets (secondary to consumption) or otherwise unexplained graft dysfunction, should also trigger suspicion.

Fortunately, most patients with preformed DSA have uneventful resolution posttransplant; even patients with class II DSA with mean fluorescence intensity greater than 10 000 by single-antigen bead analysis (One Lambda, LABScreen, US) experience resolution two-thirds of the time.[108] After transplant, the risk for de novo DSA in the first year ranges from 0% to 8%, but when it occurs, the risk of death is doubled.[4] Some risk factors for de novo DSA formation include the immunosuppression used (tacrolimus-based regimens have the lowest risk), adherence to the regimen, age of the patient and MELD score at transplant.[4]

Although acute AMR only affects ~1% of liver transplants, chronic AMR may be occurring more commonly. Several groups have reported an increased incidence of occult

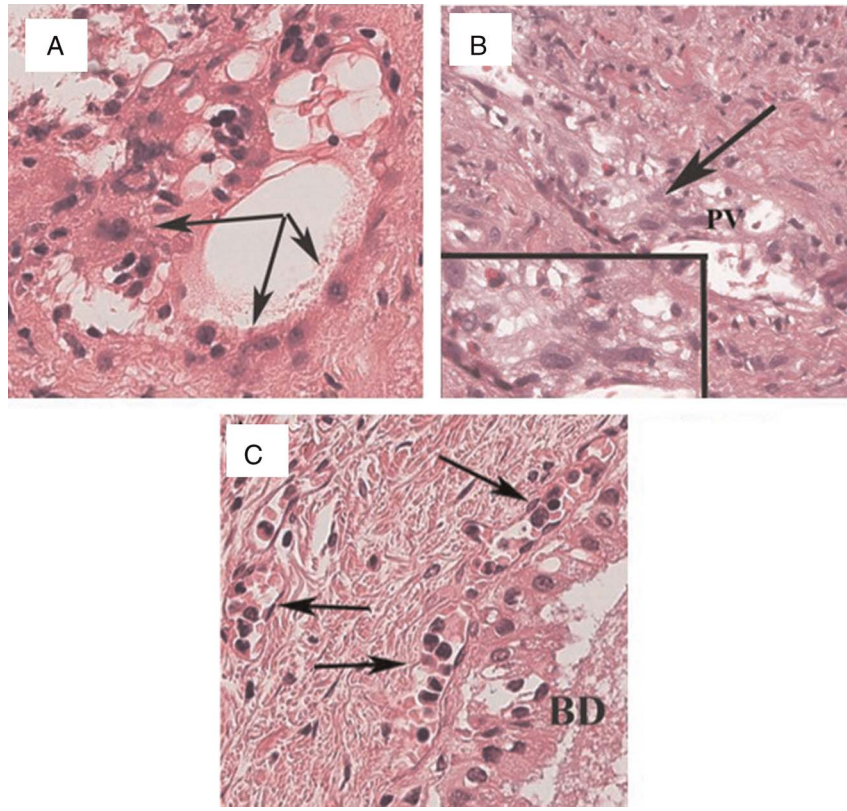


FIGURE 10. (A) Endothelial cell hypertrophy, (B) portal vein eosinophilia, and (C) eosinophilic venulitis are 3 characteristic findings in a patient with antibody-mediated rejection postliver transplantation. PV, portal vein; BD, bile duct. Figure adapted with permission from O'Leary JG, Michelle Shiller S, Bellamy C, et al. Acute liver allograft antibody-mediated rejection: an inter-institutional study of significant histopathological features. *Liver Transpl*. 2014;20:1244–1255 doi:10.1002/lt.23948.

fibrosis in the presence of posttransplant DSA.[109-116] In pediatric liver transplant, DSA+ versus DSA– patients are more likely to develop advanced fibrosis on protocol biopsy.[110] DSA+ versus DSA– HCV viremic patients had a tripling in their risk of advanced fibrosis by 1 year posttransplant.[112] In patients >6 months posttransplant, 9% of DSA–patients developed de novo DSA, one quarter experienced acute AMR, and even HCV-negative patients with DSA (versus without) had more advanced fibrosis.[109] Diagnostic criteria for chronic AMR have recently been proposed for the identification of liver allograft recipients with DSA at higher risk for allograft loss.[117]

When attempting to identify DSA+ patients at highest risk for overt problems, several factors have been elucidated. The quantity of alloantibody likely plays a role, in both preformed and de novo DSA at the population level, but, given the qualitative nature of current testing, this is difficult to pinpoint.[4,105,107,108,118] IgG3 subclass testing versus standard DSA testing better predicted the risk for death in a large, single-center, retrospective analysis of preformed and de novo DSA.[119] Further supporting this concept, in a pediatric weaning trial, some of the 12 tolerant patients had DSA but none had IgG3 DSA.[120,121] In addition, the presence of an HLA and a non-HLA DSA may increase the risk for fibrosis progression. A small pediatric liver allograft study demonstrated that patients without DSA had the lowest risk of advanced fibrosis, those with either an HLA or angiotensin II type 1 receptor (AT_1R) autoantibody had an intermediate risk, and all patients with both HLA DSA and AT_1R antibodies developed advanced fibrosis.[111] In summary, patients at higher likelihood of developing progressive problems in the face of DSA posttransplant include those with: (1) a higher quantity of HLA DSA (which is difficult to measure with today's technology); (2) IgG3 subclass-positive DSA; (3) those with an additional injury, such as HCV infection, that can upregulate class II expression in the liver and thereby facilitate binding with crosslinking to allow injury to occur[117]; and (4) possibly those with both HLA and non-HLA antibodies.

Unfortunately, at the present time, liver biopsy is still needed to determine if injury is occurring in patients with DSA in serum. However, in the future, we hope biomarkers will be used to examine patients' risk for de novo DSA formation and injury in the face of posttransplant DSA, and, more importantly, evaluate the risk of immunosuppression minimization. Although simplistic, perhaps DQ matching could help with risk stratification; patients who are DQ-matched to their donor may be at the lowest risk for de novo DSA formation and, therefore, I hypothesize, at a lower risk for immunologic complications with immunosuppression minimization, although this hypothesis requires evaluation. On the other hand, class II DSA may possibly be used as a biomarker of alloimmune reactivity, potentially indicating the need for more intense immunosuppression, although, once again, this hypothesis has never been tested and will require formal evaluation. As a result, the next step toward evaluating these hypotheses will be to implement 'standard protocol' class II DSA testing, with biopsies when positive, in all immunosuppression trials and into standard of care in centers with a research interest in further understanding the role of DSA in liver allograft fibrosis and function.

Our understanding of the impact of DSA and acute and chronic AMR on liver allografts is rapidly evolving, and we will be better positioned to predict long-term outcomes of our patients once multicenter studies with protocol DSA testing and biopsies have been performed. At the present time, immunosuppression regimens should not be adjusted based on DSA data alone. Risk stratification pretransplant can be accomplished to some degree through pretransplant DSA testing, although some do not feel this is a cost-effective approach given the low prevalence of acute AMR. However, of greater prevalence than acute AMR is the emerging concept of chronic AMR associated with posttransplant DSA and occult fibrosis progression, usually in the face of a normal or near-normal liver injury test. Ultimately, the most cost-effective approach to improving outcomes from de novo DSA is not through testing, but though prevention. Fortunately, compared with CsA-based regimens, adherence to a tacrolimus-based immunosuppression regimen is associated with the lowest risk of developing de novo DSA.[4]

Early Allograft Dysfunction and Biliary Strictures After Liver Transplantation

Prof Jacques Pirenne

In liver transplantation, 2 complications, early allograft dysfunction (EAD) and biliary strictures (BS), correlate with increased hospital stay and/or hospital readmissions, inferior graft and patient survival, and increased costs.[122] Both conditions are the end result of a cascade of tissue injuries that precede transplantation (preexisting disease in the donor, brain death-induced injury, surgical trauma, cold preservation and warm ischemia) and culminate in IRI in the recipient. By modifying these risk factors and preventing organ damage we have the potential to improve the results of liver transplantation and widen its application by increasing the pool of organs suitable for transplantation.

The incidence of EAD in liver transplant recipients is approximately 25%.[123] In a single center cohort, we found the use of imported livers, the use of histidine-tryptophan-ketoglutarate (HTK) as a preservation solution, high MELD scores, and longer cold ischemia time to be risk factors for EAD.[122] The incidence of BS in livers from deceased and living donors varies widely, from 4% to 40%.[124] Risk factors include donation after cardiac death, donor age, prolonged warm and cold ischemia time, and extended use of vasopressors in the donor.[125]

There are a variety of interventions that can be considered in the prevention of EAD and BS. Modification of these risk factors should begin during preretrieval of the organ for transplantation and continue through procurement, preservation of the organ and posttransplantation. Transplant teams classically aim to procure organs rapidly to avoid sustained brain death-induced inflammation. Recent studies in kidney transplantation suggest that delaying procurement after brain death is beneficial for organ recovery,[126] as this allows anti-inflammatory mechanisms to become activated. Whether this strategy ("relax and repair" instead of "rush and retrieve")[126] is also valid in liver transplantation is still to be confirmed. A recent clinical trial demonstrated that the use of steroid therapy in deceased donors reduces IRI and biliary injury, and improves graft function.[127] The administration of an infusion of N-acetylcysteine before and during procurement has also demonstrated efficacy in improving graft survival in liver transplantation.[128]

During procurement, organ manipulation, which can induce liver injury,[129] should be minimized. Rapid extraction is deemed necessary to prevent rewarming of the organ after perfusion, since prolonged extraction time has been linked to early graft failure in kidney transplantation.[130] The use of a double-perfusion strategy (aortic and portal flush) has been shown to be beneficial for suboptimal livers, reducing graft primary dysfunction and increasing patient and graft survival.[131] The incidence of BS has been reduced through the use of low-viscosity preservation fluids, fluid pressurization, and the addition of urokinase to the preservation solution and in the hepatic artery.[132-134]

Data from the ELTR suggest that the University of Wisconsin, Celsior and IGL-I preservation solutions perform better than HTK, the latter being associated with a 10% increase in the risk of graft loss.[135] In animal models, the addition of trophic factors to preservation solutions improves organ function immediately posttransplant.[136] In human liver transplantation, the administration of a pan-caspase inhibitor to the preservation solution has been shown to result in lower transaminase levels.[137] Cold storage may be suitable for low- and normal-risk organs, but the time taken to reach 4°C and the low, yet persistent, level of metabolism at this temperature causes tissue trauma in the absence of oxygen. However, it has also been shown that toward the end of cold storage, retrograde oxygen perfusion reduces EAD.[138]

In recent years, the field of organ preservation has been revolutionized by the development of hypothermic machine perfusion (HMP) and normothermic machine perfusion (NMP). In kidney transplantation, HMP reduces the incidence of DGF and improves graft survival.[42] In a porcine study, continuous HMP reduced hepatocyte injury but also led to an increase in Kupffer and sinusoidal endothelial cell activation, which could eventually result in poor long-term graft survival.[139] However, improved results may be achieved through the use of postcold-storage HMP.[140] The big question is whether HMP techniques will reduce the incidence of BS. Studies in pigs and rats have shown a reduction in arteriolonecrosis of the peribiliary plexus[141] and reduced intrahepatic biliary fibrosis,[142] but these results need to be confirmed in the clinic through RCTs. In NMP, the liver is kept alive ex situ by perfusion with warm oxygenated blood. A recent study used continuous NMP from procurement to transplantation in pig liver transplants, resulting in good posttransplantation survival even after 20 hours of warm preservation.[143] The use of continuous perfusion is thought to be necessary because NMP is less effective after cold storage. Ongoing trials are studying whether this strategy can reduce the incidence of EAD and BS.

Organ management in the recipient is also an important consideration when attempting to mitigate IRI. The administration of thymoglobulin to liver transplant recipients has resulted in a reduction in transaminase levels.[144] There is current uncertainty regarding whether the use of erythropoietin derivatives would be beneficial[145]; however, the use of antiselectins appears to reduce IRI after liver transplantation.[146] There is also the potential to use inhaled nitric oxide to recover liver function posttransplantation,[147] whereas another intervention that could be worth consideration is the use of mesenchymal stem cells, which have been shown to

enhance recovery from acute renal failure[148] and to protect against liver IRI in animal models.[149]

While a number of these interventions look promising in initial trials, using single compounds may not be an effective approach to prevent EAD and BS that are multifactorial in origin; instead, the use of *multiple* strategies targeting multiple mechanisms could be more useful. One study demonstrated this by targeting multiple mechanisms of IRI in pig livers donated after circulatory death. The multifactorial treatment resulted in the elimination of primary nonfunction in recipients, reduced inflammation, improved liver function and increased recipient survival. This technique also showed potential in reducing the incidence of BS, as signified by a reduction in the biliary bile salt-to-phospholipid ratio, a surrogate marker for bile duct injury.[150]

In conclusion, EAD and BS remain major risk factors for poor graft survival in liver transplantation. However, there are some known risk factors that can be adjusted and interventions that can be used to mitigate them in clinical practice. Some strategies are already available and should belong to the standard of care for our patients, and some are in development, but it is important that interventions be applied at each step of the transplantation process.

Adherence, or Lack of it, After Liver Transplantation

Prof John O'Grady

The requirement to take immunosuppressive therapy on a regular basis to maintain liver graft function is a basic assumption of clinical practice. Patient-driven nonadherence with this commitment occurs more frequently than clinicians tend to believe and can be very difficult to detect. It also may have no immediate consequence and this reinforces the practice in patient behavior. Clinicians can be blinded to deliberate nonadherence by the practice of taking medication immediately before a clinic visit. On the other hand, patients may be unaware that some aspect of their behavior that does not comply with protocol could be detrimental to their graft.

The immunosuppression regimen is specific for drug doses but also for the intervals between doses and issues such as the relationship to food. Clinicians are probably naïve in their expectation of their patients' ability to comply with this regimen and nonadherence is more prevalent than assumed.[60] The scale of deviation from the intended regimen is broad; any deviation is classified as nonadherence. The clinical consequence of this nonadherence is variable and may not directly correlate with the degree of deviation from protocol.

Detection of nonadherence relies on a combination of enquiry and investigation. Simply asking every patient how good they are at taking their medication is an important starting point. When nonadherence is suspected but not acknowledged, information may be gained from drug levels in the blood, pill counts, prescription patterns and electronic monitoring. Nonadherence varies with time of day and day of the week; morning doses are less likely to be missed and the peak time for nonadherence is Saturday evening.[67]

The profile of the nonadherent patient should be considered unpredictable, but younger patients and those with a history of substance abuse or poor life satisfaction may be at particular risk. However, the classic association is with adolescence, with some estimates being in excess of 50% nonadherence. It is considered to be a downside of maturation as the process of developing autonomy, separating from parents and assimilating with peers progresses.[151]

Education and convenience of the immunosuppressive regimen are core to the cause of promoting drug adherence. The latter is aided by the least possible number of medications and time points during the day when these need to be taken. An illustrative study demonstrated that conversion from twice-daily tacrolimus to the modified-release preparation taken once daily resulted in a more than 50% reduction in nonadherence.[152]

There are a range of responses to nonadherence once it has been identified. These generally have cognitive, behavioral and affective dimensions, as well as health care interventions. These were assessed by a meta-analysis of 12 studies that enrolled between 18 and 110 patients. No one intervention was considered superior and a combined approach seemed to be most effective. However, it was noteworthy that the health care interventions had the least impact.[153] These findings indicate that preventing the pattern of behavior that fosters nonadherence is preferable to trying to reverse deviation from disciplined practice.

There may be some ambivalence amongst clinicians regarding the importance of total adherence to the prescribed immunosuppressive regimen. This may be reflected in an approach that reflects the perceived risk of nonadherence to intervention being triggered by events such as unexplained graft dysfunction or wide variability in blood levels of immunosuppressive drugs. However, when graft function is good, this approach permits a degree of nonadherence. The more proactive approach to monitoring adherence is based on the belief that stable long-term immunosuppression is the basis for successful liver transplantation.

ACR is the most obvious consequence of nonadherence. Late ACR episodes are more difficult to treat than similar episodes occurring within the first 10 days of transplantation. There are other less certain but potential consequences of erratic immunosuppression, for example, AMR, plasma cell hepatitis and idiopathic fibrosis. These issues are deserving of further study, but in the meantime, it seems prudent to advocate stable immunosuppression strategies as the most effective protection.

Variability of Tacrolimus Exposure in Liver Transplantation

Dr Varuna Aluvihare

Data show that early posttransplant patient and graft survival continues to improve, although little progress has been made on late (>5 years) survival.[2] Watt et al[154] demonstrated that, excluding year 1 mortality, two-thirds of all-cause mortality is not directly related to the transplanted liver. The significant contribution of nongraft mortality to death in late transplantation[104] is, therefore, one of our biggest challenges.

Both interpatient and intrapatient variabilities of tacrolimus exposure posttransplant is well established, but its implications for liver transplant recipients compared with kidney transplant recipients are less well characterized. Variability of tacrolimus exposure is often determined by differences in trough levels over time, or the standard deviation around the mean, and data are available that demonstrate that tacrolimus is associated with both interpatient and intrapatient variability of exposure.[155-158]

Interpatient Variability of Tacrolimus Exposure

Interpatient variability is, in part, due to polymorphisms that affect bioavailability of tacrolimus (eg, in the cytochrome P450 system and the P-glycoprotein expression system, which impact on tacrolimus metabolism and absorption, respectively) and this is particularly relevant in certain ethnic groups.[156,157,159] Although interpatient variability is well established, data relating to its impact on clinical outcomes are scarce.

Intrapatient Variability of Tacrolimus Exposure

In our center, we studied 64 patients converted early (≤1 month) and 65 patients converted late (>1 month) postliver transplant from immediate-release to prolonged-release tacrolimus. These data were compared with 60 patients who remained on immediate-release tacrolimus. Using dose-normalized tacrolimus trough levels, lower interpatient variability was observed at all time points with early posttransplant conversion compared with late conversion or continuation on the immediate-release tacrolimus formulation. This difference was maintained over 6 months of treatment. The study also demonstrated that intrapatient variability was lower with prolonged-release versus immediate-release tacrolimus with late posttransplant conversion.[160] These data are supported by other published studies that show a reduction in variability of tacrolimus exposure with prolonged-release versus immediate-release tacrolimus.[161,162]

In a study assessing intrapatient variability in a pediatric population of 101 patients, a standard deviation greater than 2 around the mean tacrolimus trough level was associated with late allograft rejection.[163] Another study described a "medication level variability index" (MLVI); MLVI was defined as the standard deviation of tacrolimus blood levels for each patient from a minimum of 4 readings during the study period. Not only was the MLVI associated with rejection, it actually predicted it,[164] making this method a robust measure of consistency of exposure. A reduction in interpatient and intrapatient variability may, in part, contribute to the significant improvements in graft and patient survival achieved with prolonged-release versus immediate-release tacrolimus that have recently been described.[18] Unfortunately, variability of tacrolimus exposure was not formally analyzed alongside the clinical findings in the ELTR study.[18]

It is well known that nonadherence to treatment is a risk factor for poor long-term outcomes in solid-organ transplantation. Our data indicate that converting patients who have had poor medication adherence with twice-daily, immediate-release tacrolimus to once-daily, prolonged-release tacrolimus improves adherence and clinical outcomes. It is noteworthy that few clinical programs proactively monitor nonadherence. Treatment is, therefore, often only initiated when prolonged nonadherence has precipitated graft dysfunction, by which time graft damage has already occurred. Furthermore, the pattern of nonadherence is likely well established and long-lasting.

Bioequivalence of Tacrolimus Formulations

Emerging data indicate that different tacrolimus formulations may have differential effects on rejection and renal function, potentially impacting long-term outcomes. In addition to the innovator drugs of immediate-release and prolonged-release tacrolimus, generic versions of the immediate-release formulation exist. In order for the generic formulations to be available in clinical practice they have to be shown to be bioequivalent to the innovator drug. However, there is a lack of consensus as to what constitutes bioequivalence in different countries, which is of particular significance when considering drugs with a narrow therapeutic index, such as tacrolimus. Furthermore, these studies have been performed on healthy volunteers rather than transplant patients, and do not take into account differing posttransplant drug regimens, gastrointestinal function and clinical course. Given the above, the potential for unanticipated clinical events despite bioequivalence remains a concern, as does any impact on interpatient and intrapatient variability. Heightened vigilance and adequate therapeutic drug monitoring need to be used and more data on the impact of variability of tacrolimus exposure needs to be obtained.

Summary

There is an urgent need to develop tests to stratify risk for poor graft and patient survival in clinical practice. First, variability of tacrolimus exposure needs to be more effectively measured than is usual in clinical practice, where the last 2 or 3 measurements are usually considered. An electronic system that notifies transplant physicians when a patient has a high variability of tacrolimus exposure, before they present with clinical symptoms, would be optimal. Better risk stratification of nonadherence to treatment early posttransplant needs to be developed. Barriers to adherence should be examined using existing resources; for example, we use a nurse-led health promotion clinic specifically aimed at identifying and treating nonadherence. Improved characterization of patient phenotypes, including immune phenotypes defining the high-risk recipient, needs to be developed. Finally, tacrolimus formulations and drug regimens that minimize variability of exposure and improve adherence should be preferentially used.

Underimmunosuppression in Liver Transplantation

Dr Pavel Trunečka

CNIs remain pivotal to postliver transplantation immunosuppression. There is an abundance of literature comparing the 2 CNIs, tacrolimus and CsA; generally, the finding is that tacrolimus is superior to CsA in improving graft and patient survival, and preventing AR after liver transplantation.[165] In 2010, a study was published confirming the noninferiority of efficacy for prolonged-release versus immediate-release tacrolimus,[166] and since then, there has been evidence to suggest that prolonged-release tacrolimus is associated with graft and patient survival benefits for liver transplant patients compared with the immediate-release formulation.[18] Despite the unquestionable improvements in posttransplantation immunosuppression since its inception, there remain some drawbacks, especially with CNIs, including the potential for metabolic and cardiovascular complications, and renal insufficiency. A decline in renal function is thought to be one of the main causes of poor overall outcomes postliver transplant. Data from the US National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) liver transplantation database suggest that the influence of renal failure on long-term outcomes increases over time, with renal failure associated with a hazard ratio of 7.5, >5 years posttransplant.[154] Some complications affecting long-term patient survival after liver

transplantation are associated with the immunosuppressive regimens used,[167] although data from the NIDDK suggest that diligent management of modifiable factors, including diabetes, hypertension and renal insufficiency, could improve long-term patient survival.[154]

The risk of impaired renal function and the need for renal replacement therapy in liver transplant recipients is thought to be increasing due to the use of organs from ECDs and transplantation in older patients with higher MELD scores. A recent study using measured and estimated GFR (mGFR; eGFR) demonstrated that decreased renal function is associated with a trend toward increased risk of death, with that risk increasing exponentially when GFR falls below 30 mL/min per 1.73 m^2 (relative risk, 2.28-3.62).[168] Results from this study also suggest that creatinine-based calculations of eGFR underestimate the mortality risk compared with mGFR.[168]

The immunosuppressive regimen used most commonly in liver transplantation consists of tacrolimus in combination with MMF, with or without steroids. It is important to achieve the right level of tacrolimus exposure, as underexposure has been linked with reduced graft survival and patient outcomes.[169] The concept of underimmunosuppression (or overminimization) is different in kidney and liver transplantation. In kidney transplantation, chronic allograft nephropathy, once considered a sign of CNI toxicity, is caused, in part, by alloreactivity; therefore, minimization of tacrolimus exposure intensifies the deleterious effect on the kidney. This mechanism does not contribute to kidney failure in liver transplant recipients because alloreactivity, due to inadequate suppression, does not contribute to damage of the native kidney. Therefore, historically, it was thought that to reduce any CNI nephrotoxicity postliver transplant, the logical goal was to minimize exposure to CNI. Rationale for CNI minimization after liver transplantation came from 2 principal expectations that are not well supported in scientific literature. The first assumption was that exposure to CNI is the main reason for kidney failure posttransplant and the second was that ACR after liver transplantation does not harm the graft in the long term.

In terms of exposure, lowering the exposure to CNI can be achieved by dose minimization or delaying the administration of CNI until several days posttransplantation. Data from the ReSpECT study showed that patients receiving reduced-dose (target trough level, ≤8 ng/mL), immediate-release tacrolimus delayed until day 5, in combination with daclizumab, MMF and corticosteroids had less kidney impairment after 52 weeks compared with those receiving a higher-dose (target trough level, >10 ng/mL), immediate administration of the same formulation of tacrolimus (without daclizumab and MMF).[170] It was unclear from the results of the ReSpECT study whether the reduced renal impairment was due to the delayed administration of tacrolimus or the overall reduction in exposure over time. The DIAMOND study (ADvagraf studIed in combinAtion with MycOphenolate mofetil aND basiliximab in liver transplantation) was designed to investigate administration of prolonged-release tacrolimus-based regimens further. The study design was similar to that of the ReSpECT study, with 857 liver transplant recipients receiving MMF and a single bolus of intraoperative corticosteroids, in combination with one of the following: standard-dose, prolonged-release tacrolimus (target trough level, 5-15 ng/mL until day 42 then 5-12 ng/mL); lower-dose, prolonged-release tacrolimus (4-12 ng/mL until day 42 then reduced by 20-25%) and basiliximab; or standard-dose, prolonged-release tacrolimus (5-15 ng/mL until day 42) delayed until day 5 and basiliximab.[171] Results from this study indicated that lower-dose, prolonged-release tacrolimus administered immediately posttransplant (and a subsequent lower tacrolimus exposure over the first month), together with MMF and basiliximab, was associated with a significant renal function benefit and a significantly lower incidence of biopsy-confirmed acute rejection (BCAR) versus the standard-dose, prolonged-release tacrolimus-based regimen (Figure 11).[171] Delayed initiation of standard-dose, prolonged-release tacrolimus significantly reduced renal function impairment; however, the BCAR incidence advantage was not seen with delayed initiation (Figure 11).[171] Results from this study indicate that early tacrolimus exposure in the immediate posttransplant period may be critical in maintaining renal function over the long term.

An old view of ACR as a harmless or even protective event promoting tolerance and long-term graft survival supported the strategy of CNI overminimization.[172] In light of the possibly deleterious effect of the association between DSA and under immunosuppression, views regarding the role of ACR changed; ACR is now less acceptable and this new perspective is supported by published literature.[105,106,108] In a study of 493 patients, those with tacrolimus trough levels greater than 7 ng/mL, measured on the day of protocol biopsy, experienced fewer moderate/severe rejection episodes during the first 2 weeks posttransplant compared with those with tacrolimus trough levels 7 ng/mL or lower (Figure 12).[169] Data from this study indicate that trough levels of 7 to 10 ng/mL are efficacious in preventing AR and are associated with longer-term graft survival compared with patients whose trough levels are outside that window.[169] A systematic

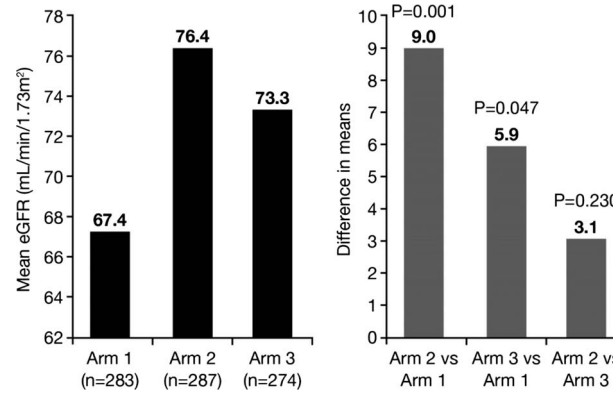


FIGURE 11. Renal function (eGFR (MDRD4)) at week 24 with different prolonged-release tacrolimus-based regimens in liver transplant patients. Data are based on the full-analysis set. *P* values were analyzed by ANOVA. Arm 1: standard-dose, prolonged-release tacrolimus plus mycophenolate mofetil (MMF); Arm 2: lower-dose, prolonged-release tacrolimus plus MMF and basiliximab; Arm 3: standard-dose, prolonged-release tacrolimus (delayed until day 5) plus MMF and basiliximab. ANOVA, analysis of variance; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; MMF, mycophenolate mofetil. Adapted with permission from Trunečka P, Klempnauer J, Bechstein WO, et al. Renal function in de novo liver transplant recipients receiving different prolonged-release tacrolimus regimens—the DIAMOND study. Am J Transplant. 2015;15:1843–1854 doi:10.1111/ajt.13182. Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

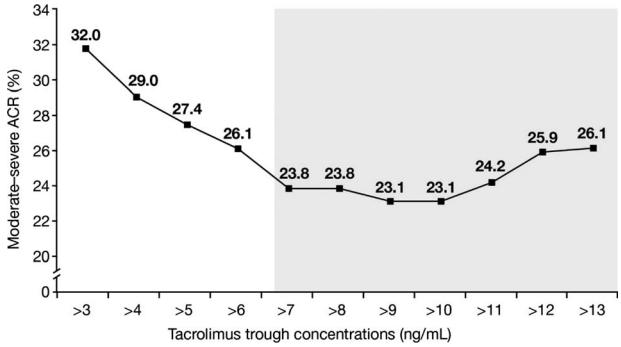


FIGURE 12. Incidence of moderate/severe histologic rejection in protocol biopsies of transplant recipients stratified by tacrolimus trough concentration. Shaded box shows trough concentrations ≥7 ng/mL. Tacrolimus was administered immediately posttransplant (0.1 mg/kg per day in 2 divided doses), after which the dose was adjusted according to blood trough concentrations and renal dysfunction or other side effects. Protocol biopsies were performed within the first 15 days posttransplant. ACR, acute cellular rejection. Reprinted with permission from Rodríguez-Perálvarez M, Germani G, Papastergiou V, et al. Early tacrolimus exposure after liver transplantation: relationship with moderate/severe acute rejection and long-term outcome. *J Hepatol.* 2013;58: 262–270 doi:10.1016/j.jhep.2012.09.019.

review of 64 liver transplant studies suggested that tacrolimus trough levels between 6 and 10 ng/mL during the first 4 to 6 weeks after transplantation could reduce renal impairment without increasing the incidence of moderate/severe ACR.[173]

Early immunosuppression seems necessary for optimal immunologic outcome in liver transplant recipients, especially in light of the role of de novo DSAs and the association between DSAs, AR and graft loss. The concept of tacrolimus minimization as a protective factor in liver transplantation is not based on evidence or data from RCTs in liver transplantation, but seemingly on traditional views of CNI-related side effects (predominantly on kidney function). The role of ACR in liver transplantation must be reevaluated, as the implications seem to have a greater impact on long-term outcomes than traditionally believed. Maintenance steroids have been found to be generally unnecessary for the avoidance of ACR in liver transplantation.[171] Immunosuppressive protocols should avoid early alloimmune reactivity; optimal tacrolimus trough levels for a liver transplant recipient early posttransplant are in the range of 6 to 10 ng/mL, with limited evidence for any beneficial effects of delayed administration of tacrolimus after transplantation in standard recipient populations.

Cardiovascular Complications in Liver Transplantation

Dr Umberto Baccarani

Liver transplantation is associated with an increase in cardiovascular risk, ranging from 9.4% at 5 years to 25% at 10 years.[174] Cardiac evaluation before liver transplantation is essential to decrease mortality and to prevent new cardiovascular diseases posttransplant. The most common pretransplant cardiac condition affecting patients with liver cirrhosis is cirrhotic cardiomyopathy. In addition, left ventricular outflow tract obstruction, coronary artery disease (CAD), portopulmonary hypertension and NODAT are frequently recorded.

Cirrhotic cardiomyopathy is a cardiac dysfunction in patients with advanced liver disease, characterized by impaired cardiac contractility and altered diastolic and systolic function with electrophysiological abnormalities.[175] From the clinical point of view, it is characterized by hyperdynamic circulation with increased cardiac output secondary to low systemic vascular resistance and increased arterial compliance. The potential impact of cirrhotic cardiomyopathy is related to the severity of liver disease, ending in cardiac failure when the patient presents with end-stage liver disease and decompensated cirrhosis.

Plotkin et al[176] reported a 50% incidence of mortality in liver transplant recipients with a history of CAD (1- to 3-year follow up). In patients over 50 years of age, a 27% incidence of moderate or severe CAD was reported. Diabetes was the most important predictive risk factor for CAD.[177] An abnormal noninvasive test or a high pretest probability of CAD (≥2 classical risk factors) is an indication for coronary angiography or cardiac computed tomography angiography.[178]

Patients with left ventricular outflow tract obstruction may exhibit a poor tolerance to hemodynamic stress during transplant[179] and careful intraoperative monitoring with trans-esophageal echocardiography is required to avoid tachycardia. Limited use of inotropic agents and trans-esophageal echocardiography-guided volume administration is advisable to avoid overload of the right ventricle.

Hypertension is a primary consequence of immunosuppression and of renal disease. Recommendations for patient management comprise blood pressure monitoring and early-onset treatment, as in the general population; for patients without proteinuria, anti-hypertensive therapy should be initiated with a calcium channel blocker. Many patients require combination therapy, with the addition of an angiotensin II receptor blocker or angiotensin-converting-enzyme inhibitor. Early withdrawal of steroids posttransplant and monitoring/modification of the immunosuppression regimen is also recommended.

Metabolic syndrome has been described in 44% to 58% of patients after 6 months posttransplant and it is associated with increased cardiovascular and cerebrovascular events,[180] mainly due to an increased prevalence of metabolic syndrome features (diabetes, hypertension, and hyperlipidemia) posttransplant.[180]

Portopulmonary hypertension may affect 5% to 10% of liver transplant candidates, resulting in poor long-term outcomes. Before liver transplantation, patients need to be treated with prostanoids, phosphodiesterase inhibitors and endothelin receptor antagonists to modify this risk factor for poor patient survival. The reported frequency of hepatopulmonary syndrome in patients with liver disease ranges between 4% and 29%.[181] The treatment of hepatopulmonary syndrome includes the correction of hypoxemia by administration of oxygen. Patients with hepatopulmonary syndrome typically have normal or only mildly elevated pulmonary arterial pressure, and liver transplant may be curative.

NODAT has been recognized as a clinically important complication in liver transplantation. A stepwise treatment to NODAT considers nonpharmacological therapy with lifestyle modification plus education.[182] If glycemia is not controlled, monotherapy with an oral anti-diabetic medication is considered. If individualized goals for glucose control are not achieved in 2 to 4 months, a reassessment of lifestyle interventions is required in addition to oral combination therapy.[182] Many studies have described the high prevalence and incidence of dyslipidemia in patients posttransplant.[183,184] Immunosuppressive treatment could affect lipid metabolism.[180] Annual screening of a patient's lipid profile, with treatment

thresholds and targets based on those advocated for the high-risk population, is recommended. For patients not responding to dietary interventions, statins are recommended as first-line agents. Therapy modifications are suggested for patients not responding to drug therapy, including the conversion from CsA to tacrolimus.[185]

Van Wagner et al[186] analyzed the early cardiovascular mortality of 1576 liver transplant recipients who died within 30 days of transplant. Using a logistic regression model, the authors were able to develop a predictive model of early cardiovascular mortality, finding 9 main predictors: 6 recipient covariates (age, preoperative hospitalization, intensive care unit requirement, ventilator status, MELD score and history of portal vein thrombosis), 2 donor covariates (national organ sharing, donor body mass index) and 1 operative covariate (cold ischemia time).

Cardiovascular events are one of the most important causes of morbidity and mortality postliver transplant. In pretransplant patients, attention should be placed on identifying subclinical cardiac events that influence early and long-term outcomes. In patients with portopulmonary hypertension, screening with transthoracic echocardiography, confirmed by right heart catheterization, and treatment before liver transplant are recommended. Screening for CAD is recommended for high-risk patients. Given poor sensitivity and negative predictive value of noninvasive tests, coronary angiography is recommended in the presence of >2 risk factors. Transthoracic echocardiography before transplant, for screening, and intraoperative trans-esophageal echocardiography during transplant, for close hemodynamic surveillance, are key examinations to monitor cardiac function. There is an ongoing need for further research, potentially using large-scale, real-world data sets, to continue to identify the specific risk factors for the development of cardiovascular complications posttransplant, and for these risk factors to be prospectively managed, where possible, before the occurrence of a cardiovascular event.

Lifestyle modification is currently the first approach to reducing cardiovascular risk. When therapeutic changes are proven to be ineffective, modification of the immunosuppressive regimen and specific medication should be considered.

PART 2: SOLUTIONS AND INNOVATIONS WITHIN KIDNEY AND LIVER TRANSPLANTATION

Long-Term Patient Management

Optimizing Resource Allocation in Transplantation

Prof James Neuberger

Solid organ transplantation is expensive in terms of time, expertise and other associated resources; the imbalance between need and availability of organs means there has to be rationing of an intervention that improves both longevity and quality of life. The limited data available suggest that, in some countries at least, both volume and location of transplant units could affect access to, and outcomes of transplants.[187-189] However, transplant units have often developed in an unplanned manner.

A national approach to patient selection and organ allocation is necessary to ensure an equitable use of resources, although variation in acceptance rates of organs between centers and surgeons may lead to inequity of access to transplantation. Monitoring of absolute and risk-adjusted outcomes from both listing and transplantation is necessary to ensure good governance and the best use of resources. However, care must be exercised so that monitoring does not encourage inappropriate risk-averse behavior or inhibit research, innovation or training. Optimizing resources, especially of donated organs, therefore requires consideration of both the commissioning of transplant units and monitoring the outcome of donated organs.

Provision of Transplant Units

An efficient national transplant service requires an effective program to ensure an adequate supply of donated organs, accurate donor and organ characterization, an effective retrieval service, and the provision of transplant units that operate in an ethical and legal framework with appropriate levels of regulation, oversight and transparency. Each transplant unit requires a skilled multidisciplinary team of surgeons, physicians, intensivists, anesthetists, interventional radiologists, histopathologists, pharmacists, nurses, dieticians, and coordinators. They must also be supported by specialists in infectious diseases, and alcohol and substance abuse. The provision of transplant services should further be based on need, available resources and geography; but in practice, units have developed because of the enthusiasm of clinicians, and are supported by hospital managers who recognize the financial and reputational benefits of a successful unit. Provision of organs for transplantation and routine care in transplant units vary considerably (Table 2), as do the outcomes achieved.[190,191]

Studies are conflicting as to whether transplant volume has an effect on outcomes. There are more data relating to center transplant volumes for liver transplant than for other organs. An analysis of nearly 35000 liver transplant recipients in the ELTR showed patients transplanted in centers performing 70 or more transplants/year had significantly better early outcomes compared with patients transplanted in centers performing less than 70 transplants/year.[192] Another earlier study found that outcomes were better in patients transplanted in centers performing more than 20 transplants/year versus less than 20 transplants/year, whereas, a study by Nijboer et al[193,194] did not find a clear correlation between center activity and outcomes in 24 liver transplant units in Germany. In a similar US-based study, Macomber et al[195] found lower mortality rates in higher-volume centers, with lower median length of hospital and intensive care unit stay and lower direct costs. Others have failed to demonstrate an effect of volume.[196,197] The variation in conclusions as to the impact of volume on outcomes could relate, at least in part, to the impact of small numbers, the relative bluntness of risk adjustment, the fact that some small-volume centers may be led by very experienced surgeons trained elsewhere, and the experience with related surgical interventions.[198] These figures, however, do not take into account other activities, such as living or deceased donors, liver resection and other solid organ transplants. In conclusion, there is a suggestion that, for optimal outcomes, a center undertaking adult deceased-donor liver transplants should be performing at least 20 per year, without evidence to suggest an upper limit beyond which outcomes decline.

Geography and the Provision of Transplant Units

The provision of services is affected by geography, so distribution of units must reflect, in part, the population

TABLE 2.

Distribution of liver and kidney transplant units

Country	Liver transplant units	Liver transplants per million population	Liver transplant units per million population	Kidney transplant units	Kidney transplants per million population	Kidney transplant units per million population
United States	139	19.8	0.44	250	53.2	**0.79**
Spain	25	23.2	0.53	44	47.3	**0.94**
Germany	25	13.4	0.30	40	35.9	**0.49**
France	22	18.3	0.35	44	44.7	**0.68**
Italy	22	16.4	0.36	43	28.2	**0.72**
UK	7	13.0	0.11	27	44.0	**0.44**
Switzerland	3	13.0	0.39	6	37.7	**0.77**
Ireland	1	10.9	0.22	1	37.8	**0.22**

Data from Newsletter, Transplant International Figures on Donation and Transplantation.[188,189]

distribution.[199] Geography also plays a major role in access to transplant services and outcomes, but the interaction is complex and is affected by access to specialists, referral to transplant units and variation in listing.[187,199,200] This variation could reflect other factors, such as socioeconomic status, ethnicity and burden of illness.[201-206] Whether distance from a transplant center affects the chances of getting a transplant is uncertain,[206,207] but travelling to a distant center may not always benefit the patient.[208]

Does Choice Benefit the Patient?

Opinion is divided as to whether a competitive market would increase choice and standards and drive down costs. Several studies have suggested that competition among providers of liver transplantation has mixed effects: in the studies, centers with greater competition had a higher rate of graft failure and patient death, used more high-risk grafts and had longer waiting lists, but transplanted more of the sickest patients and a greater proportion of the population (although the latter was not statistically significant).[209,210]

Can Resource Allocation Ensure Equity of Access and Outcomes?

In this context, equity means that patients with the same characteristics (age, sex, geographical location, ethnicity, lifestyle, financial resources, and disease and disease severity) will have the same chance of being listed and being offered a graft (or dying on the list), and will have similar outcomes. There is no single measure of outcome, or consistency of what outcome measurements are based upon, with some based on outcome measurement of the patient and some on outcome measurement of the graft. There is also lack of consistency with respect to assessment initiation, with some starting at the time of the patient being listed for transplantation, and others starting posttransplantation. Outcome measurement, therefore, requires risk adjustment, but this is a blunt tool and a failure to include all relevant data can produce misleading conclusions.[211]

Overreliance on outcomes in transplantation may encourage risk-averse behavior.[211,212] Surgeons concerned about adverse outcomes might be more likely to avoid circumstances that could reduce their patients' survival rates, including the avoidance of high-risk donors and recipients, not letting less-experienced colleagues and trainees develop expertise, and even a resistance to innovation. Avoiding high-risk donors will increase the risk of death without a transplant, and death while awaiting a transplant is just as lethal as death posttransplant. A higher survival rate could, therefore, come at the price of lower benefit. Publication of outcome data alone could lead to distorted conclusions and could drive clinical practice against the interests of the patient. Thus, patients should be given information, both absolute and risk-adjusted, about the outcomes for each center from listings and from transplantation. While this seems a clear goal and is carried out both in the UK (www.odt.nhs.uk) and the USA (www.unos.org), it is practiced in few other jurisdictions.

Is National Allocation of Donated Organs the Best Use of Resources?

There are complex algorithms for allocation of organs for transplantation. In the United States, as in many other jurisdictions, livers are allocated primarily on the basis of need. This has been effective in reducing the mortality of patients on the waiting list, but potentially at the expense of increasing posttransplant resource utilization,[213] and sometimes excludes from access those with conditions that make life intolerable (intractable severe itching or chronic encephalopathy). Kidney allocation tends to be more complex than liver allocation, based on multiple factors, including waiting time, blood group, sensitization, and age matching.

Both organ allocation and graft outcome monitoring must not inhibit innovation and research. There is limited evidence to suggest that centers supporting clinical research deliver better clinical care; however, a national allocation scheme based on need is confounded by variation in acceptance rates.[213-215] The appetite for risk-taking varies not only between, but also within units,[210] and may be affected by overzealous outcome monitoring.[212] Furthermore, the ability to predict the donor-specific risk of graft failure is relatively poor, with a systematic bias toward inaccurately low estimates of graft failure, especially for higher-risk organs.[216] Allocation schemes are, in reality, offering schemes and because centers vary in their use of organs, reliance on allocation cannot achieve the best use of resource.

Clinical Innovation in Transplantation

Dr Philip F Halloran

The very success of organ transplantation presents us with a host of problems and opportunities for improvement. There are approximately 120000 new organ transplants per year worldwide (available at: http://issuu.com/o-n-t/docs/2012ad. Accessed 15 March 2016) and approximately

one million people with organ transplants, but many are troubled and are in danger of failing. There are hundreds of thousands of people on transplantation waiting lists and millions more who could potentially benefit from organ transplantation. Meeting these challenges will require clinical innovation, and part of the problem is the high level of organ transplant attrition. Tens of thousands of organ transplants fail each year and many more organs function suboptimally. Immunosuppressive regimens face the challenge of nonadherence to treatment, which is responsible for the high rate of graft failure involving AMR,[57] and of delivery of affordable drugs in populations where the cost of drugs is challenging.

Innovation is needed to provide more organs to meet demand, new diagnostic techniques to identify the disease states, a reduction in the burden of immunosuppression toxicity, and strategies to prevent and treat the main causes of graft loss, including AMR, nonadherence to treatment, and recurrent disease. Examples of how research could impact these needs include the optimization of donor organ use (and using currently discarded organs if possible), molecular phenotyping, tolerance initiatives to reduce the need for immunosuppression, and regenerative medicine applications to create, engineer or repair organs for transplantation. Investment from the pharmaceutical and biotechnology industry will be critical, because grants from governments and altruistic agencies are unlikely to be sufficient.

Improved utilization of donor organs must include more effective identification and conversion of potential deceased donors. We need more evidence to support the choices made to use or discard ECD organs, avoiding the loss of useful organs at a time when the need is so pressing. Molecular phenotyping probably offers the greatest opportunity to develop that evidence. Measurements must address both the degree of acute organ injury and the extent of biologic aging.

Opportunities to improve the availability of organs for transplantation may be found in ex vivo perfusion, such as the remarkable advances in ex vivo lung preservation,[217] which has resulted in the use of many lungs that would have previously been discarded. The use of discarded organs as scaffolds shows promise and some groups continue to explore the potential applications of xenotransplantation, especially using genetically modified pig organs. The prospect of monetary rewards for donation is an issue that should be discussed, despite the deep divisions in opinion.

Precision medicine generally requires assessments that identify heterogeneity in the disease states. This will require molecular phenotyping, as has been widely used in cancer.[218] Molecular biopsy interpretation can create new disease classification, recalibrate the conventional methods, correct errors, and reveal mechanisms that can be targeted by new drugs. Improved disease classification will empower the search for noninvasive biomarkers in body fluids, which has been limited by inaccuracies in the current diagnostic systems. To serve as screening tests, potential biomarkers must be inexpensive and superior to existing tests, such as urine dipstick testing for protein and serum creatinine. Distinguishing abnormal from normal is usually done well by the current screening tests. The ideal biomarker should distinguish specific disease states from nonspecific inflammation and injury.

Molecular genetics present some possibilities for insight and innovation. For example, there are research groups using genotyping to study rare donor and recipient mismatches, such as the homozygous loss of a protein in the recipient only, which could lead to an immune response to the donor organ. High-resolution tissue typing may be able to predict which mismatches are at high risk for triggering destructive DSAs.

Tolerance research is based on the idea that the naturally occurring negative regulatory mechanisms in the immune response can be exploited to achieve graft survival free from immunosuppressive drugs. The assumption is that early interventions will provide durable, long-term changes in the immune system that protect patients from TCMR and AMR. However, TCMR and AMR are independently regulated. TCMR is rare after 5 years, with virtually no cases after 10 years posttransplant, apparently representing time-dependent partial adaptive tolerance that precludes TCMR. In contrast, AMR cases began to present approximately 1 year posttransplant and continued to present even 30 years posttransplant.[12] Tolerance initiatives need to take into account the separate regulation of AMR and TCMR and demonstrate that the intervention can reliably prevent both, even late AMR. Clinicians also need to reflect on some key issues associated with conditioning protocols and cell injections in tolerance studies, such as their safety and durability.

More information about the immunologic events and immune regulation in the prevalent transplant population on immunosuppression would be welcome and would synergize with tolerance initiatives. What is the effect of immunosuppressive drugs on long-term adaptive changes and regulatory circuits? Are many patients currently not at risk and receiving more immunosuppression than they need? What phenotypes represent nonadherence, and what phenotypes represent the inherent failure of the current immunosuppressive drugs to control late AMR?

Regenerative medicine holds promise for reconditioning of old organs and the generation of new ones. However, any solutions must be robust, durable and able to compete with biomechanical alternatives. There are also technical barriers to consider, such as our limited ability to preserve organs on normothermic ex vivo perfusion for prolonged periods, and the inherent limitations in somatic cells, such as replicative senescence, autophagy, mTOR mechanisms and other aging mechanisms. We must also consider how regenerative medicine strategies, dependent on many cycles of cell division, will impact the risk of oncogenesis and senescence.

In conclusion, the unmet needs in organ transplantation present us with many opportunities for clinical innovation. We have listed some, but there are many others. Transplantation has always been inspired by the dream that clinical innovation can change patient outcomes, and that spirit must be renewed if the next generation of challenges is to be met.

Annual Review Clinic—and Other Models for the Future

Mr Marc Clancy and Prof Alan Jardine

Posttransplant follow-up care is a series of consultations through which we aim to maximize patient and graft survival, and quality of life. In the early period after transplantation, the focus is on prevention of rejection episodes and the optimization of graft function. In the longer term, however, we need to pay attention to the long-term risks of an otherwise successful transplant, which include an increased risk

of infection, malignancy and premature cardiovascular disease. There is also a need to reinforce the need for life-long adherence to medication. The purpose of this short review is to highlight some issues and strategies to optimize long-term treatment.

In Scotland, and elsewhere in the US and Europe, there are 2 models for transplant follow-up: a "centralized" model and a "repatriation" model. In the "centralized" model, patients return to the transplant center and are seen by dedicated transplant specialists on an ongoing basis. In the "repatriation" model, the transplant and early follow-up care is carried out in the transplant center, and the patient is then "repatriated" to their original referring unit for their follow-up care, usually by nephrologists with more limited transplant expertise. The East coast of Scotland uses the "repatriation" model and the West coast uses the "centralized" model of care. The difference is driven largely by population demographics, and there is similar variation in other regions of the world. For example, Israni et al[219] recently reported significant variation in the structure and processes of postkidney transplant care in the United States.

Data from the Scottish Renal Registry show a steep upward trend in the number of patients with end-stage renal failure, the majority of whom are now transplant recipients (Figure 13).[220] This is a pending capacity issue in terms of future posttransplant follow-up, the potential shortage of nephrologists with transplant experience, and the need to design robust posttransplant follow-up models for the future.

An annual review clinic is one component of this follow-up strategy. This involves a multidisciplinary assessment to focus on factors that may not be a priority at routine clinic visits. These include the identification and management of risk factors (eg, for cardiovascular disease, infection or malignancy), and provision of health promotion advice and support for both patients and relatives. It is part of a wider strategy to promote patient engagement and empowerment, and to improve adherence to treatment. There is no single perfect model for posttransplant care, but there are numerous national and international advisory guidelines.[221,222]

A typical structure for an annual review would involve skin surveillance, most commonly by a dermatologist and involving photography; a review of cardiovascular risk (fasting lipids, diabetes, blood pressure and medications, such as statins); screening for diabetes (fasting glucose, HbA1c or glucose tolerance test); and a discussion about adherence. For some patients, advice, on diet and weight loss may be required, for others, specific issues, such as prepregnancy advice, may be needed.

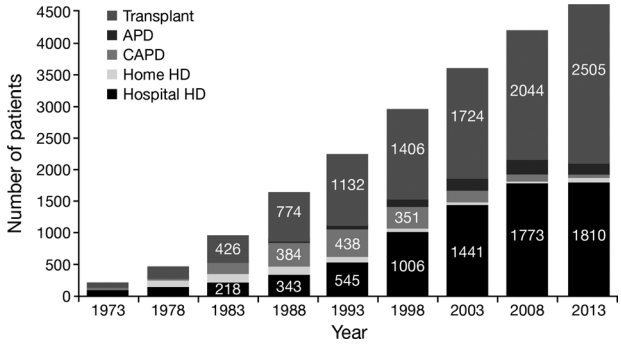


FIGURE 13. Maintenance renal replacement therapy trends in Scotland. APD, automated peritoneal dialysis; CAPD, continuous ambulatory peritoneal dialysis; HD, hemodialysis. Reprinted from NHS National Services Scotland. Scottish renal registry report. http://www.srr.scot.nhs.uk/Publications/Main.html. ISD Scotland.

Medicine has evolved from a traditional paternalistic model to a more collaborative approach. Patients have ready access to medical information and to their own clinical data. The clinical consultation is also more bi-directional than in the past. In the US, there is an online portal, Renal Patient View (www.patientview.org), where patients can view their results, medications, and letters sent about them to other health care professionals. Patients can also enter blood pressure, glucose and weight measurements taken at home. Renal Patient View has been adopted by many clinics and patients, and its uptake has been rapid and extensive. Egton Medical Information Systems (https://patient.emisaccess.co.uk. Accessed 15 March 2016) is another system accessible by patients that is widely used across primary health care in the US. These web-based systems, accessible through a variety of platforms—including smartphones—give patients ownership of their own care and dynamic access to laboratory data. The use of such technology allows us to monitor patients remotely, in real-time, in a way that was previously unthinkable, while empowering patients to take a much more active role in their own management with respect to both adherence and risk-factor management.

There are tools that have been developed to help identify patients at risk of poor long-term outcomes, including a 'cardiovascular risk calculator' for renal transplant recipients.[98] This calculator enables quick estimation of a patient's cardiovascular risk in the clinic, and estimates the potential benefit of, for example, weight loss or lipid lowering therapy.[98] Another tool is the 'risk factor calculator' app (Astellas Pharma Europe Ltd). This app enables assessment of adherence using the validated BAASIS questionnaire,[223] evolution of renal function by linear regression, using previously collected eGFR (MDRD), and estimation of intrapatient variability of tacrolimus exposure. The use of this type of calculator with the patient can help explain the importance of risk management and is likely to be an emerging technology for the future.

The rate at which the posttransplant population is growing is not being reflected in the resources available to manage these patients, suggesting that capacity may become an issue within clinics in the future. Moving some monitoring of patient parameters outside of the conventional clinical setting may be a viable option. A number of remote patient monitoring and telehealth devices are already in use, where measurements, for example, blood pressure, hemoglobin, glucose, HbA1c and pill taking, and so on, can be captured at home and automatically transmitted back to the clinic. Telemedicine is being piloted in transplantation follow up to monitor nonadherence to treatment.[224]

As we develop strategies for the future, we need to focus on the management and risk of the emerging complications—especially cancer and cardiovascular disease—and the risks of complacency and nonadherence. An annual review clinic with a multidisciplinary team to assess cardiovascular risk, diabetes, dermatology (skin surveillance for malignancies), cardiology, medication adherence, graft surveillance and biopsy—clinical measurements beyond the typical simple consultation—is one strategy. Similarly, technological advances may allow us to empower and educate patients, and even monitor them remotely, to improve long-term graft and patient outcomes without overburdening clinical services.

Clinical Innovation in Medicine

Prof Edward K Geissler

Preventing immunologic rejection of transplanted organs without the need for long-term use of pharmacological immunosuppression is a primary objective in transplantation medicine. Reducing this need would dramatically improve the outcome for transplant recipients and would reduce health care costs. A reliable means of achieving this goal has not been realized with pharmacologic or biologic agents, so we must now look to new, innovative approaches. With this objective, The ONE Study consortium of European Union-led investigators and colleagues from the United States are applying the concept of cell therapy to human kidney transplantation.

Conditioning the immune response of transplant recipients toward allograft acceptance, using advanced, cell-based medicinal products, is a promising therapeutic approach, and is now becoming technically feasible. There are various alternative tolerance-promoting cell types, including regulatory T cells, macrophages and dendritic cells, which are now at a state of development that allows them to be trialled in early-stage clinical studies. The central focus of The ONE Study is to produce distinct populations of hematopoietic regulatory cells and, simultaneously, comparatively test their safety and promise in minimizing pharmacologic immunosuppression in organ transplantation.

To assess this, several regulatory cell products are being tested in a coordinated group of clinical trials through The ONE Study; they have been licensed over the past few years for production according to stringent manufacturing practice conditions. By directly evaluating the various immunoregulatory cell therapies against one another in this way, and also against a group of patients not receiving cell therapy (Reference Group Trial), The ONE Study is expected to determine whether further testing of each cell product in clinical trials is warranted. We have chosen to include in The ONE Study those cell preparations that, in our view, represent the most promising therapeutic agents, and which are ready for clinical application: M regs (Regensburg); polyclonal Tregs (Charité, Berlin, and King's College London and the University of Oxford); donor-antigen-reactive Tregs (the University of California, San Francisco); donor alloanergized Tregs (Massachusetts General Hospital, Harvard University, Boston); Tr1 cells (Fondazione Centro San Raffaele, Milan); and tolerogenic dendritic cells (CHU de Nantes).

During the first period of the project, the main activity of the clinical centers involved in The ONE Study has been the preparation of the clinical protocols for the Reference Group Trial and the submission of an IMPD (Investigational Medicinal Product Dossier) or IND (Investigational New Drug) to the regulatory agencies for the Cell Therapy Trials, as well as the development of a centralized immune monitoring program. The Reference Group Trial (www.clinicaltrials.gov) is being conducted at all of the sites mentioned above, and serves as an independent, multi-center, phase IV clinical trial that is designed to corroborate historic renal transplantation statistics and generate 'baseline' immunologic health-related quality of life, and health-economic data sets for The ONE Study cell therapy trials. The Reference Group Trial uses a standard immunosuppressive treatment regimen that is similar to the one that is being used in the Cell Therapy Trials. The Reference Group Trial has finished recruitment, and has now entered the follow-up phase. In parallel, 6 Cell Therapy Trials have begun and will continue to recruit patients, the goal being to recruit 8 to 16 patients into each trial. All of the groups conducting Cell Therapy Trials will essentially follow the same immunosuppressive treatment regimen, with the exception that their specific cell therapy products will be applied to the kidney transplant recipients; cell infusion replaces basiliximab induction therapy being used in the Reference Group Trial. With The ONE Study concept, it will be possible to compare the results of each cell therapy trial to each other, as well as with the results from patients receiving standard therapy in the Reference Group Trial.

One of the objectives of the ONE Study is to standardize immune monitoring of transplant recipients, so that a true and fair comparison of results can be made between the individual cell therapy trials, as well as between those trials and the Reference Group Trial. The ONE Study immune monitoring concept involves using strict standardized sample collection and testing (eg, flow cytometry, polymerase chain reaction, and so on). Testing is performed centrally at the Charité (Berlin) to ensure that the test results from all samples collected in the different trials, and at the different sites, can be reliably and meaningfully compared. The immune monitoring has already been fully developed and implemented in the Reference Group Trial, demonstrating the feasibility and value of this testing concept. For instance, The ONE Study flow cytometry panels have been developed and standardized with a third party (Beckman Coulter).[225] The concept of The ONE Study immune monitoring has generated significant interest and is presently being adopted by other international immune monitoring laboratories as a means of standardizing immune monitoring performance.[226] Standardized electronic data collection systems have also been developed in parallel to ensure consistent data gathering. This conceptual approach to immune monitoring is a major accomplishment of The ONE Study.

In summary, the ONE Study is progressing well toward the planned objectives. The Reference Group Trial, to which the Cell Therapy Trials will be compared, has completed recruitment. Moreover, 6 Cell Therapy Trials have begun and remain in the recruitment phase. Supporting these studies, our novel immune monitoring program has been fully developed, standardized and implemented successfully in this logistically challenging international study, providing a conceptual framework for other clinical trials that could benefit from precise monitoring of immunologic therapies. The ONE Study is well underway and is expected to yield useful information regarding the potential for cell therapy in kidney transplantation as a means of reducing the need for conventional immunosuppressive drugs.

Clinical Innovation in Transplantation

Dr Alejandro Soto-Gutiérrez

End-stage liver disease is responsible for over 30 000 deaths annually in the United States alone.[227,228] Medical therapy can prolong life, but the only definitive therapy for severe cases of end-stage liver disease is allogeneic liver transplantation—either a partial liver from a living-related donor or a whole deceased-donor liver. The success of liver transplantation has evolved in many ways, and indications for this therapeutic modality have expanded to include many

causes of acute and chronic liver failure, cirrhosis, inherited metabolic diseases, and some cancers.[229,230] Yet, the pool of donor livers fails to keep pace with the growing demand.

Based on the US OPTN registry data (https://optn.transplant.hrsa.gov/), as of 10 July 2015, there were 15 751 people on the liver waiting list in 2014, and only 6729 of these received a transplant. In addition to this, there is also an expectation that the donor pool will shrink further due to the obesity epidemic. The use of donor livers with greater than 30% liver steatosis is increasingly common in transplantation, the presence of which is a significant risk factor for increased primary nonfunction and initial poor graft function in liver transplantation.[231] These data point to one fact: organ availability is an absolute constraint on the number of liver transplants that can be performed. Thus, creation and universal availability of a liver graft from autologous tissue and cells (eg, induced pluripotent-derived liver cells) would dramatically change this equation by increasing the number of organs available for transplantation, and eliminating the need for life-long immunosuppression and its accompanying complications.

The ongoing shortage of available organs has led investigations toward innovative transplant techniques, including efforts to produce implantable grafts without the need for an organ donor. A popular strategy being developed for the creation of such implantable liver grafts involves the decellularization of whole organs, and subsequent reseeding with relevant cell types, followed by maturation of the neo-organ in a physiologically appropriate bioreactor, and then implantation of these constructs in animals.[232-240] Although most studies using this approach have demonstrated feasibility, they have also demonstrated limited survivability and function of the grafts after implantation into animals. These disappointing results have been largely attributed to a failure to maintain a durable vascular network and failure to rebuild the complex liver microarchitecture required for function by the assembled liver graft after transplantation. Currently, the major challenge with regard to the success of transplantable bioengineered liver grafts is the development of durable vascular networks. Any incompletely reendothelialized vasculature is at risk for acute thrombosis, leading to localized organ failure. Therefore, ideally, complete vascular endothelialization is desirable for clinical applications; however, new nonthrombogenic biomaterial technologies could provide a useful alternative.[241,242]

With regard to the development of autologous liver grafts, the identification of an abundant source of human cells is a major limitation to the clinical application of bioengineered organs for transplantation. To date, induced pluripotent stem cells (iPSCs) represent a potential source for liver tissue creation. Nevertheless, complete reestablishment of the liver microarchitecture would require incorporation of liver nonparenchymal cells (eg, bile duct cells, sinusoidal endothelial cells, stellate cells, and so on), necessitating iPSC differentiation for these cell types. Additionally, differentiated cells derived from iPSCs may still be immature, functionally resembling fetal or neonatal phenotypes. Thus, cell maturity may prove to be critical for diseases requiring functional differentiated cells. In some instances of end-stage liver disease, and for inborn errors of liver metabolism, an entire liver may not be required and auxiliary partial liver transplantation has been suggested as a therapeutic option,[243] although a graft representing at least 35% of a liver would be necessary.

Another major challenge in the field of liver engineering will be to design protocols for massive cell engraftment to fulfil the necessary liver mass for transplantation. Organ-engineering technology is in its infancy and will need to overcome countless translational hurdles before liver grafts can be used widely in preclinical studies. However, most elements of the technology, although not yet validated by a reproducible protocol, are in place. Critical aspects of the developmental biology of the liver remain unknown, but the manufacture of replacement organs is no longer science fiction and remains a promising area for investigation.

Technological Innovation in Transplantation

Prof Christophe Mariat

There are a number of limitations facing the transplantation community; however, identifying these inadequacies enables us to translate limitations into innovations, leading to improved outcomes for our patients. The phenomenon of the emergence of DSAs, and the central role played by humoral immunity in the process of graft failure, led to the introduction of Luminex-based antibody screening technology into clinical practice, which has revolutionized diagnostic procedures in transplantation and has led to a greater understanding of the causes of graft failure. Many other technologies are also being developed and are set to further improve the way we diagnose and treat our patients; however, these technologies are currently in their infancy and are not yet available for routine clinical practice. The development of innovations that were not necessarily originally focused on health care, including mobile applications, are also under way to facilitate, and in some cases improve, the interaction between patients and their physicians. These innovations include the ingestible sensor system (ISS), a care/monitoring application, and a microsampling device.

The ISS is being developed to accurately track patients' adherence to treatment by the ingestion of microsensors with, or included in, the oral dosage form of drugs.[244] This system has already been introduced in Europe and the United States.[244] The ISS consists of an ingestible event marker (IEM), the microsensor that becomes activated after ingestion, and an adhesive personal monitor patch to detect the IEM once activated. Using wireless Bluetooth-based technology, the adhesive personal monitor can relay information to a smartphone, which in turn sends the data to a secured, centralized data storage and processing location. Although this "Big Brother" style approach may seem daunting, the system has been associated with high (99.4%) adherence to mycophenolate in kidney transplantation,[244] and similar innovations are already being used in various fields of medicine. As adherence to treatment is particularly important in transplantation, these innovative techniques could help improve long-term outcomes for our patients.

The patient care/monitoring application is being developed to help manage and provide appropriate care for the growing number of transplant outpatients. Such patients typically only attend an annual hospital consultation; however, between these yearly visits they must also undergo routine biologic check-ups in a laboratory closer to their home. This means that the laboratory must send the information to the physician, who must then compare results with the patient's medical chart review to make an accurate analysis. This

process is time-consuming and often inefficient. The introduction of a care/monitoring application could help improve the efficiency of this process and could easily be implemented in the clinic. The patient can download the application to their smartphone, enter the value of their test results in the application themselves, and send messages to the physician. In turn, the physician will receive these results on his/her computer. A "flag" appears on the physician's interface for any patient who has results outside the normal range that has been specifically set for that patient. The physician can then assess the reasons for the red flag and implement the appropriate action. For transplant patients, this innovation can be used to monitor variables, such as serum creatinine levels, hemoglobin concentrations, leukocyte counts, and trough levels of immunosuppressive drugs.

This type of patient monitoring system is currently being implemented in several centers, including the Nephrology, Dialysis and Renal Transplantation Center at Jean Monnet University. So far, 50 patients have used this system and the feedback has been positive, with patients frequently commenting that they feel safer using this type of technology to measure their health status. One drawback, however, is the overutilization of the text messaging option, with physicians being inundated with questions that would ordinarily be addressed by a general practitioner. Further assessment of the system on a larger scale is warranted to determine its impact on the physicians' workloads and on patients' treatment outcomes.

The third type of innovation in development is a microsampling device to help with therapeutic drug monitoring in transplant outpatients. There are several certified immunoassays that can be used for therapeutic drug monitoring, depending on the laboratory used. As a result, variability is observed between readings from 1 laboratory versus another (for example between external and central laboratories), based on the assay used. When comparing results between centers this should, therefore, be taken into account.[245,246] The introduction of a microsampling device could help circumvent this issue: a patient, with the assistance of a nurse, if required, can collect samples at home using a ready-to-use chip and forward them to the laboratory for analysis. This is similar to available methods for monitoring blood glucose concentrations in patients with diabetes. With such a system, therapeutic drug monitoring could be performed using a single drop of blood, which could be sent for analysis at a central laboratory. Initial validation of the system for tacrolimus measurements have shown promising results in our clinic, with strong correlations between tacrolimus concentration readings from the device and readings from traditional blood samples. Further analytic and clinical validation of the system is required before it can be introduced into routine clinical practice.

In conclusion, various technologies and innovations to improve outcomes for transplant patients are currently in development. A major focus is on applications to improve adherence to medication, although there is limited information regarding the impact of such applications on clinical outcomes. A primary concern with the advent of such applications is the ability to maintain patients' privacy, particularly if applications are somehow connected to social networking sites. In addition, it is important that such innovations are tailored to meet the needs of both patient and physician, and that both adapt their routines or habits to ensure that the introduction of these novel technologies is effective and beneficial to both parties.

Transforming Health Care: the Patient and the App

Mr Monty Metzger

The evidence of the past is all around us and information we create immediately becomes history. The past provides a great resource of knowledge, and human nature generally results in a backward-looking society. Perhaps only now we are beginning to understand how this past information can help us to create an understanding of what the future might hold. The story of the patient and the app illustrates how digitalization is shaping the evolution of the health care industry.

The future of health care is fast approaching: a future where, instead of coming in for routine check-ups, your patients' clinical indicators will be collected using wearable technologies, sent securely to your clinic, and video consultations will be used to discuss their recent readings. A future where complete medical records and histories will flow seamlessly between the relevant members of the collaborative health care team and results will be displayed in context with the wider patient population. The demands on the health care system have been getting incrementally larger and change, although daunting, is essential. Glimmers of this change are starting to appear.

Digitalization advantageously combines 4 unique factors: (1) a global talent and knowledge-sharing community where patients and experts collaborate online, and can all benefit from virtually inexhaustible resources; (2) low production costs—a smart team can often develop a digital product in only a few hours; (3) lean technology—innovation through trial and error rather than attempting to produce a 100% perfect product the first time around; and (4) new access to capital—for example, funding available through potential patients or "crowdfunding," where products that have not even been produced or introduced can be funded by their target audience.

There are indications that these 4 elements are coming together to address health care issues. Venture capitalists from Silicon Valley and New York invested over 6 billion US dollars in health technologies in 2015. The fact that many were start-up companies implies that a significant paradigm shift toward technological development has occurred.

Whether it is a revolution led by Twitter or transportation services like Uber, digital influences are shaping all industries. Within health care, we are experiencing a dramatic change in the way we produce and consume data. The smartphone in particular liberates and empowers us to a new degree; for digitalized health care and medicine, it is the biggest moment of opportunity in our lifetimes. The digitalization of routine medicine will allow patients to shop by price, convenience and reputation, and to benefit from a greater number of options. What does this mean for physicians and how will the health care community evolve to continue to communicate with the digitally empowered patient? As we transition toward the doctorless patient and virtual clinic visits/consultations, a 40% reduction in doctors' visits resulting from "virtual visits" represents a 60 billion dollar market opportunity in the United States alone.

The empowered patient can be armed with substantial knowledge about their lifestyle and conditions. For a 200 US dollar price tag, one can already purchase a number of

consumer devices: Kito (by Azoi Inc.), for example, plugs into an iPhone to measure metrics such as blood pressure, electrocardiography data, blood oxygen, temperature and lung function; Scanadu Scout is a similar crowdfunded project. One can obtain an interactive pregnancy tracking device to track heart rate or fetal rollovers, or a '23andMe' kit that provides considerable amounts of genetic data.

Empowered patients typically participate in social communities to gather advice or self-diagnose before medical intervention. They take a central role in determining when they interact with the health care system and how their care is delivered. They are better prepared to collaborate with their doctors on the best course of action, and help implement treatment plans to ensure optimal outcomes. Advanced learning centers and the development of algorithms are providing more information about these patients. This information is used to assist the patient to make healthier lifestyle decisions, but the potential to pair the data with gaming mechanics could also help to provide services to insurance companies, for example. The net result of this approach may be a healthier population that is less reliant on the resources provided by the broader health care system.

Wherever we are, our smartphones and personal devices provide us with abundant information. Many tools, such as Fitbit and Jawbone, exist to allow an individual to measure vital signs and fitness data, and there are global online wellbeing communities, such as Quantified Self, which promotes itself using the slogan 'self-knowledge through numbers'. Within the hospital environment too, there is a trend toward ubiquitous information. Philips are currently exploring the potential of Google Glass to allow doctors to acquire critical information about their patients in a hands-free format.

With many of the new technologies, the challenge is really how to use and visualize the data collected, a fact that also pertains to many big data projects that are frequently being undertaken in medicine. For example, projects that use sensors within contact lenses to measure glucose metrics, or those using ingested sensors to monitor internal factors have constant data streams to be processed. These remote methods of data collection are also reshaping the relationship that has traditionally defined health care: the relationship between the patient and the doctor.

In essence, our ability to successfully orchestrate the information from our past with the real-time data that we are collecting today could culminate in predictive technology that can give us better insights into our future. One thing in our future is certain already, though: everything in the future that can be digitalized will be digitalized, and the rest will be as well.

Thinking Big: "Big Data" and the Future of Health Care

Sir Muir Gray

Health care has made an astonishing impact on the health of populations and individuals in the last 50 years, and transplantation has been an example of the way in which high-tech science has been translated into effective care. This has been the second revolution in health care—the first being the Public Health Revolution of the 19th century. However, at the end of 50 years of astonishing progress, a number of problems can be seen in every country and these were revealed by the unwarranted variations that are a striking feature of health care.

Unwarranted variations were defined by John Wennberg, who published the first Atlas of Health care (available at: http://www.dartmouthatlas.org/. Accessed 15 March 2016) from Dartmouth University, as variations that cannot be explained by either variation in need or variation in preference.[247] With the exception of trauma, and to a lesser extent cancer, unwarranted variation can be observed in every clinical specialty in conditions where only the people who are affected reach the specialized services. An example of this, the variation in prostate-specific antigen testing in different populations, is shown in Figure 14.

The unwarranted variation reveals other problems.

- Failure to prevent disease and disability (for example, failure to prevent stroke among people with atrial fibrillation)
- Waste of resources
- Inequity (under provision of services to some groups)
- Overuse and harm resulting from what the BMJ has called "Too Much Medicine" (available at: http://www.bmj.com/too-much-medicine. Accessed 15 March 2016)

All of these have been revealed by big data.

Big Data

The term "big data" is so widely used that it requires definition and there are, of course, many definitions. In this paper we are using "big data" to refer to the management of health data in what might be called 'cloud computing' rather than mainframe computing. For years we have struggled to connect different mainframes, but now the internet has changed that and allows us to relate data much more easily. The consequence of this has been a shift from a focus on institutional quality to a focus on value.

Quality and Value

It is vitally important to improve the quality and safety of care, and transplant services have demonstrated this excellently. However, quality is different from value and there are 3 aspects of value set out below:

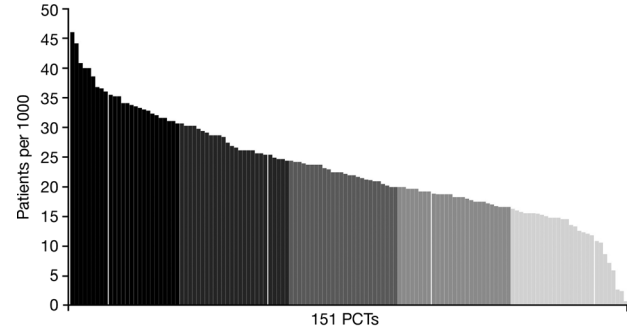


FIGURE 14. The variation in prostate-specific antigen testing in different populations. Shading represents the quintiles, to encourage the jurisdictions to think about overuse or underuse of diagnostic services, if they appear in the top or bottom quintile. PCT, primary care trust. Reprinted from NHS England. The NHS Atlas of Variation in Diagnostic Services. Reducing Unwarranted Variation to Increase Value and Improve Quality. November 2013.

- Allocative value, determined by how assets are distributed to different subgroups in the population
- Technical value, determined by how well resources are used for all the people in need in the population
- Personalized value, determined by how well decisions relate to the values of each individual

The technical quality of transplant services is high, based on registries, but in thinking about the future for both kidney and liver transplantation we need to think about 2 other aspects that are interlinked: allocative and personal aspects of value.

Allocative Value and Transplantation

Over the last 30 years, investment in health services has increased steadily, but, partly as a consequence of the global financial collapse, the growth is stopping in many countries. In the countries in which it is continuing, the gap between need and demand on the one hand and resources on the other, will place intense pressure on budgets. Furthermore, need is increasing in both kidney and liver disease, with the latter presenting a number of big challenges. Obviously, a case could be made for increased liver transplantation, but the increase in obesity and HCV also demand attention and resources. This means that within each of these conditions choices have to be made.

Within liver disease, the choices include:

- Increased investment in HCV prevention
- Increased investment in HCV treatment
- Increased investment in the prevention of obesity
- Increased investment in prevention of alcohol misuse
- Increased investment in end-stage liver failure and transplantation

These choices have never been starkly presented before, but are now the focus of a major research effort in Oxford, based at the Institute for Value-based Health Care. For kidney disease, the choices are between the different modalities of managing kidney disease—prevention, treatment, dialysis and transplantation—and it may be that resources need to be switched from dialysis to transplantation to take into account personalized value.

Personalized Value

Decisions made by individuals relate obviously to the evidence, but that evidence has to be tailored to their particular clinical condition by the clinician. The third factor in a decision is their values. Personalized Value and Value for Populations, both allocative and technical, are interwoven. At a population level there is great concern about what Avedis Donabedian proposed in 1980 as care beyond the point of optimality, illustrated in Figure 15. In this figure, it can be seen that investment reaches a point at which the balance of benefit to harm is not suitable for society[249]; antibiotic prescribing is a classic example of this. However, there is growing concern about people receiving inappropriate dialysis when they have multiple conditions. It may be that if choices were put to individuals with a number of comorbidities they would choose not to have dialysis, thus freeing up resources, which can be switched to transplantation.

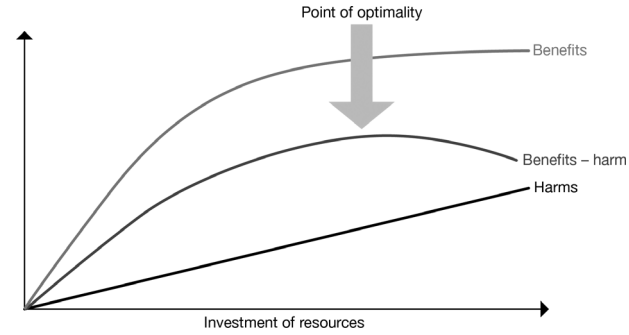


FIGURE 15. Diagrammatical representation of resource investment as a relationship to the benefit/harm ratio. Figure adapted with permission from Gray JA. Tools for Transformation: Essential Glossary for Understanding Value and Efficiency in Health and Healthcare. 3rd ed. BVHC; 2014. Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

Resource Stewardship

These are issues not for payers to consider directly, but for clinician and patient groups to discuss and debate. Furthermore, the right balance between different interventions for liver and kidney disease will differ from 1 population to another depending on the past investment decisions. These have rarely been explicit and have led to the current position in which there is a high rate of variation.

Publication of an Atlas of Organ and Transplantation is being planned by Public Health England and it will undoubtedly reveal not only wide variation, but also many opportunities for increasing value. Big data makes this possible and relatively easy for the first time.

SUMMARY OF KEY LEARNINGS

Associate Prof Jonas Wadström and Prof Bo-Göran Ericzon

The face of health care is continuously evolving and we are now on the brink of a new era of digitalized medicine. Mobile applications, such as ingestible sensor systems and patient monitoring applications are set to enter the field of transplantation. New technologies can facilitate personalization and will hopefully help us to further improve the management of transplant patients.

As we attempt to reduce the disparity between organ availability and patients awaiting transplant, we are transplanting organs from a greater number of marginal donors. Concurrently, advances are being made in donor management before procurement, as well as new technological advances in organ storage and reperfusion. Although a number of these advancements are not yet widely available, they yield promising results that could bring advances in solid organ transplantation.

Advances in transplantation, however, are not restricted to technological innovations and donor management. Our understanding of the field has been enhanced and is changing the way we treat our patients. Over the past decade, an important milestone in improving long-term outcomes has been to better understand the modifiable risk factors for graft loss. We need to look beyond the 'traditional' risk factors of age and hypertension during the management of our patients and recognize that underimmunosuppression or overimmunosuppression, poor adherence to therapy, and high variability of CNI exposure are important aspects that need to be monitored and

addressed in routine clinical practice. As we continue to extend graft survival posttransplant, we must also recognize the impact that cardiovascular disease has on the survival of transplant recipients and take steps to modify the associated risks.

With these risk factors in mind, a key unmet need currently facing the transplant community is the requirement to improve long-term transplant management in an era where there are few new phase II and III clinical trials. To achieve this, it is necessary to use new data sources to classify patients accurately and then to confirm those patients 'at risk' of poor outcomes at the individual patient level. These data assessments could lead to future tests, interventions and clinical trials. For these reasons, we are looking beyond RCTs in transplantation and using registries to give us an indication of patterns in long-term outcomes.

Summary of Key Learnings in Kidney Transplantation

Associate Prof Jonas Wadström

In light of the use of marginal donors in kidney transplantation, new techniques are required to optimize donor management, improve organ preservation after retrieval and attenuate reperfusion to improve graft survival. However, there is a gap in the literature between experimental research addressing IRI and data that can be translated to clinical practice.[43] It is clear that to improve graft and patient survival, donor management strategies need to be used within the context of patient management and alongside pharmacologic therapies.

CNI minimization, with the aim of reducing nephrotoxicity, was traditionally common practice in kidney transplantation; however, data suggest that underimmunosuppression has a negative impact on graft survival.[25,78] Registry data show that tacrolimus trough levels less than 5 ng/mL 1 year posttransplant are associated with inferior graft survival, indicating that low tacrolimus trough levels should be avoided.[25] Steps need to be taken to modify the risk factors associated with underimmunosuppression, including improving adherence to treatment and minimizing the variability of CNI exposure to improve long-term outcomes.

Data suggest that DSA now supersede CNI toxicity and chronic allograft nephropathy in their ability to cause chronic deterioration of the transplanted graft.[50] Information to assist us in understanding the intricate nature of the histologic patterns that can be indicative of subclinical AMR and kidney graft failure is emerging.[54] The implementation of effective screening procedures and improved kidney allocation policies in our clinics will enable us to move to prevent rather than treat AMR and to improve therapeutic outcomes. The development of DSA and, subsequently, the emergence of AMR posttransplant have been associated with nonadherence to treatment.

Nonadherence is widespread in the transplant community. Addressing issues associated with nonadherence remains a key challenge in transplantation, in part, due to the difficulty in assessing its prevalence, as there is currently no 'gold standard' for use in routine clinical practice. Innovative technologies have the potential to assist us in improving adherence and outcomes for our patients.[244,250] However, these technologies are not yet widely available in clinical practice.

Nonadherence to treatment has been associated with high intrapatient variability of exposure, both of which can lead to poor long-term outcomes.[30,31,57,58] High intrapatient variability of tacrolimus exposure defines a group of patients proven to manifest rejection, graft failure and dysfunction at a higher rate than the lower variability population. Although it is widely accepted that graft and patient survival can be affected by CNI trough levels falling vastly outside of the target range, the clinical impact on patients with small fluctuations outside of the therapeutic range also appears to be important. Interventions to reduce variability would, therefore, seem justified and sensible.

In order to identify patients who are at risk of nonadherence to treatment and high intrapatient variability, we need to incorporate routine monitoring of these risk factors into clinical practice. Only by identifying patients who are at risk early posttransplant and monitoring risk over time can we adjust immunosuppressive regimens and patient care to meet individual needs. Using new digital innovations, such as mobile apps and ingestible sensors, has the potential to assist us with this task without increasing the burden on our health care systems.

As long-term graft survival increases, we need to look toward the risk factors associated with cardiovascular disease to further improve survival in kidney transplant recipients. Interestingly, in a study of over a million adults (nontransplant), a decline in renal function was associated with an increased risk of the occurrence of cardiovascular events.[94] Cardiovascular risk factors should be managed both pretransplant and posttransplant and it is important to recognize the impact of the choice of immunosuppressive regimen on cardiovascular risk. As such, immunosuppression should be chosen and amended in accordance with each patient's clinical profile. Risk factor equations, with appropriate validation, could provide insights into the long-term potential of different treatment regimens and provide useful tools for individual patient counselling.[98]

As health care needs change, we should explore and embrace new technologies for patient monitoring and communication. By using technology to promote patient engagement and provide tools to enable patient empowerment, we can begin to implement these changes and to improve long-term outcomes for our patients.

Key learnings in kidney transplantation

- Information to assist us in understanding the intricate nature of the histologic patterns that can be indicative of subclinical AMR and kidney graft failure is emerging
- Nonadherence is widespread in the transplant community and this remains a key challenge
- High intrapatient variability of CNI exposure defines a group of patients who will manifest rejection, graft failure and dysfunction at a higher rate than the lower variability population
- Underimmunosuppression increases the development of DSA and AMR and has a negative impact on graft survival
- As long-term graft survival increases, we need to look toward the risk factors associated with cardiovascular disease to further improve survival in kidney transplant recipients
- By identifying "at risk" patients and modifying the risk factors for poor graft survival, we could achieve better outcomes for our patients in the long term
- As health care needs change, we should explore and embrace new technologies for patient monitoring and communication. By using these technologies to promote patient engagement and provide tools to enable patient

empowerment, we can begin to address these changes and to improve long-term outcomes for our patients

Summary of Key Learnings in Liver Transplantation

Prof Bo-Göran Ericzon

The most commonly used immunosuppressive regimen in liver transplantation consists of tacrolimus in combination with MMF, with or without steroids. Five years ago, a study was published confirming the noninferiority of efficacy with prolonged-release versus immediate-release tacrolimus,[166] and since then, there has been evidence to suggest that prolonged-release tacrolimus is associated with graft- and patient-survival benefits for liver transplant patients compared with the immediate-release formulation.[18] The reasons for the potential improvements in survival with prolonged-release tacrolimus are yet to be fully understood; however, we theorize that by using prolonged-release tacrolimus in liver transplantation, we are modifying a number of risk factors for poor graft survival, which play an important role in improving outcomes for our patients.

Risk factors, such as early allograft dysfunction and biliary strictures, remain a major cause of inferior outcomes in liver transplantation, and new techniques are required to prevent their occurrence.[122] In addition to advances in perfusion strategies, delaying organ procurement after brain death, using steroid therapy in deceased donors and using *N*-acetylcysteine before and during procurement warrant further investigation. By preventing organ damage, we can improve the results of liver transplantation and widen its application by increasing the pool of organs suitable for transplantation. Some strategies are already available and should become the standard of care for our patients; some are in development, but it is important that multiple strategies targeting multiple mechanisms are applied at each step of the transplantation process.

Pretransplant and posttransplant DSA formation presents a further challenge for improving long-term outcomes in liver transplantation. Our understanding of the impact of DSAs, and acute and chronic AMR, is still evolving. The occurrence of de novo DSA in liver transplantation is low; however, its occurrence is associated with double the risk of death.[4] This presents a particular challenge for the screening of patients for de novo DSA, making the most cost-effective approach to improving outcomes not only through testing but also through prevention. In the future, utilization of biomarkers will aid us with identification of patients at risk of de novo DSA formation and injury, and thereby guide us with regard to appropriate immunosuppression minimization.

The concept of tacrolimus minimization as a protective factor in liver transplantation is only partly based on evidence or data from RCTs in liver transplantation, but also seemingly on traditional views of CNI-related side effects (predominantly on kidney function and posttransplant lymphoproliferative disorder). It is important to achieve the right level of tacrolimus exposure both early posttransplant and during maintenance therapy, as underexposure has been linked with reduced graft survival and patient outcomes[169] and overimmunosuppression can also lead to poorer outcomes. Variability of tacrolimus exposure posttransplant is well established, but its implications for liver transplant recipients are less well characterized than for kidney transplant recipients, even though high variability of tacrolimus exposure has been associated with late allograft rejection after liver transplantation.[163,164]

High intrapatient variability of tacrolimus exposure has also been associated with nonadherence to treatment.[251] It is important to recognize that the immunosuppression regimen is not only specific for the drug dose, targeted to predefined trough levels, but also for the intervals between doses. Clinicians are probably naïve in their expectation of their patients' ability to comply with treatment regimens; nonadherence is more prevalent than assumed.[60] Perhaps emerging innovative technologies will assist us in reducing nonadherence in our patients. ACR is the most obvious consequence of nonadherence, with late acute episodes being more difficult to treat. These issues are deserving of further study, but in the meantime it seems prudent to advocate stable immunosuppression strategies as the most effective protection.

Cardiovascular events are one of the most important causes of morbidity and mortality postliver transplant. In pretransplant patients, attention should focus on identifying subclinical cardiac events, as well as screening for and treating portopulmonary hypertension and CAD. Lifestyle modification is the first approach to reducing cardiovascular risk; however, when these are proven to be ineffective, modification of the immunosuppressive regimen and specific medication should be considered. It is the choice of immunosuppressive therapy, both early posttransplant and in the long term, that still represents the most important factor influencing the posttransplant cardiovascular complications in the transplanted patient.

Through greater understanding and advances in transplantation, patients with functioning grafts are living longer, increasing the need to use health care resources more efficiently. We should learn from other therapy areas, such as diabetes, how to use new patient-orientated technologies to improve patient–physician interactions. Exploring such innovations for transplantation could enhance patient engagement, communication and treatment monitoring, and potentially translate into improvements in long-term outcomes.

Key learnings in liver transplantation

- Five years ago, non-inferiority of efficacy for prolonged-release versus immediate-release tacrolimus was reported; since then, there has been evidence to suggest that prolonged-release tacrolimus is associated with graft- and patient-survival benefits for liver transplant patients compared with the immediate-release formulation
- Early adequate immunosuppression seems necessary for optimal immunologic outcomes, especially in light of the role of de novo DSAs and the association between DSAs, AMR and graft loss
- High intrapatient variability of tacrolimus exposure has been associated with late allograft rejection
- ACR is the most obvious consequence of non-adherence to treatment, with late acute episodes being more difficult to treat
- Cardiovascular events are one of the most important causes of morbidity and mortality posttransplant
- By identifying 'at risk' patients and modifying the risk factors for poor graft survival, we could achieve better outcomes for our patients in the long term

- New patient-orientated technologies could enhance patient engagement, communication and treatment monitoring, and potentially translate into improvements in long-term outcomes

Conclusions

The field of transplantation is evolving, and early graft and patient survival rates have been on the increase across indications. However, graft and patient survival beyond the first year have shown a more modest improvement over the last decades. By routine monitoring and modification of the risk factors associated with poor graft and patient survival with the immunosuppression regimens that we use, we can intervene to improve long-term outcomes for our patients posttransplant. Only by aggressively tackling these risk factors pretransplant and posttransplant will we help to achieve better outcomes in the longer term for our patients.

With the advent of immunosuppressive therapies, including tacrolimus, we witnessed a revolution in the treatment of transplant patients. However, advancement in transplantation should not stop there; new advances in our understanding of the field, innovative technology, novel therapeutics and changes in the way research is conducted—using registries as well as RCTs—will provide insights into how we can move forward and further enhance the outlook for patients in need of transplantation. Future technologies in areas such as organ engineering also provide us with the potential to decrease our reliance on life-long immunosuppression and increase the number of organs available for transplantation. As we enter this new era, we must harness the information we have generated, reevaluate how we treat patients and ensure that necessary changes are rapidly incorporated into routine clinical practice.

More specifically, as we move toward a more personalized approach to transplantation and redefine what "best practice" treatment looks like, our ability to integrate all available and multidimensional data, including phenotypic, histopathologic, transcriptomic and immunologic information, will prove critical in identifying appropriate treatment approaches for different patients. Only with this concerted effort can we continue to make a difference to our patients' lives.

- As we enter the era of personalized and digitalized medicine, we must harness the information we have generated, reevaluate how we treat patients and ensure that necessary changes are rapidly incorporated into routine clinical practice
- Advances in our understanding of the field, innovative technology, novel therapeutics and changes in the way research is conducted—using registries as well as RCTs—will provide insights into how we can move forward and further enhance the outlook for patients in need of transplantation
- By routine monitoring and modification of the risk factors associated with poor graft and patient survival with the immunosuppression regimens that we use, we can intervene to improve long-term outcomes for our patients posttransplant

ACKNOWLEDGMENTS

The authors would like to thank Dr Alexandre Loupy for undertaking the role of Guest Editor for this supplement. They would also like to thank Nina Kennard, Amy MacLucas and James Wallis from iS LifeScience for their editorial support.

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Prescribing information and adverse event reporting information

ADVAGRAF™ 0.5 mg, 1 mg, 3 mg and 5 mg Prolonged-release hard capsules (tacrolimus) PROGRAF™ 0.5 mg, 1 mg and 5 mg hard capsules (tacrolimus)

Presentations: ADVAGRAF Prolonged-release hard capsules containing tacrolimus 0.5 mg, 1 mg, 3 mg and 5 mg PROGRAF hard capsules containing tacrolimus 0.5 mg, 1 mg and 5 mg. **Indications:** ADVAGRAF and PROGRAF: Prophylaxis of transplant rejection in adult liver or kidney allograft recipients and treatment of allograft rejection resistant to treatment with other immunosuppressive medicinal products. **Posology and Administration:** ADVAGRAF and PROGRAF therapy require careful monitoring by adequately qualified and equipped personnel. Either drug should only be prescribed, and changes in immunosuppressive therapy initiated, by physicians experienced in immunosuppressive therapy and the management of transplant patients. Dosage recommendations given below should be used as a guideline. ADVAGRAF or PROGRAF are routinely administered in conjunction with other immunosuppressive agents in the initial post-operative period. The dose may vary depending on the immunosuppressive regimen chosen. Dosing should be based on clinical assessments of rejection and tolerability aided by blood level monitoring. To suppress graft rejection immunosuppression must be maintained so no limit to the duration of oral therapy can be given. The daily dose of ADVAGRAF capsules should be taken once daily in the morning with fluid (preferably) water at least 1 hour before or 2-3 hours after a meal. PROGRAF capsules should be taken as for ADVAGRAF, in two divided doses. ADVAGRAF: In stable patients converted from PROGRAF (twice daily) to ADVAGRAF (once daily) on a 1:1 (mg:mg) total daily dose basis the systemic exposure to tacrolimus for ADVAGRAF was approximately 10% lower than for PROGRAF. The relationship between tacrolimus trough levels (C_{24}) and systemic exposure ($AUC_{0\text{-}24}$) for ADVAGRAF is similar to that of PROGRAF. When converting from PROGRAF capsules to ADVAGRAF trough levels should be measured before and within two weeks after conversion. In *de novo* kidney and liver transplant patients $AUC_{0\text{-}24}$ of tacrolimus for ADVAGRAF on Day 1 was 30% and 50% lower respectively, when compared with that for the immediate release capsules (PROGRAF) at equivalent doses. By Day 4, systemic exposure as measured by trough levels is similar for both kidney and liver transplant patients with both formulations. Race: In comparison to Caucasians, black patients may require higher tacrolimus doses to achieve similar trough levels. **Prophylaxis of transplant rejection** – liver and kidney: Initial dose of ADVAGRAF and PROGRAF capsules is 0.10-0.20 mg/kg/day for liver transplantation and 0.20-0.30 mg/kg/day for kidney transplantation starting approximately 12-18 hours for ADVAGRAF and 12hrs for PROGRAF after completion of liver or within 24 hours of completion of kidney transplant surgery. Dose adjustment post-transplant: ADVAGRAF and PROGRAF doses are usually reduced in the post-transplant period. It is possible in some cases to withdraw concomitant immunosuppressive therapy leading to ADVAGRAF monotherapy or PROGRAF dual therapy or monotherapy. Post-transplant improvement in the condition of the patient may alter the pharmacokinetics of tacrolimus and may necessitate further dose adjustments. Dose recommendations – Conversion to ADVAGRAF. Patients maintained on twice daily PROGRAF requiring conversion to once daily ADVAGRAF should be converted on a 1:1 (mg:mg) total daily dose basis. Following conversion, tacrolimus trough levels should be monitored and if necessary dose adjustments made. Care should be taken when converting patients from ciclosporin-based to tacrolimus-based therapy. Initiate ADVAGRAF after considering ciclosporin blood concentrations and clinical condition of patient. Delay dosing in presence of elevated ciclosporin blood levels. Monitor ciclosporin blood levels following conversion. Dose recommendations – Rejection therapy. Increased doses of tacrolimus, supplemental corticosteroid therapy and introduction of short courses of mono-/polyclonal antibodies have all been used. If signs of toxicity are noted the dose may need to reduced. For conversion to PROGRAF, treatment should begin with the initial oral dose recommended for primary immunosuppression. For conversion of kidney and liver recipients from other immunosuppressants to once daily ADVAGRAF, begin with the respective initial dose recommended for rejection prophylaxis. In adult heart transplant recipients converted to ADVAGRAF, an initial oral dose of 0.15 mg/kg/day should be administered once daily in the morning. For other allografts, see SPC. Therapeutic drug monitoring: Blood trough levels for ADVAGRAF should be drawn approximately 24 hours post-dosing, just prior to the next dose, for PROGRAF approximately 12 hours post-dosing. Frequent trough level monitoring in the early transplant period is recommended, with periodic monitoring during maintenance therapy. Monitoring is also recommended following conversion from PROGRAF to ADVAGRAF, dose adjustment, changes in the immunosuppressive regimen, or co-administration of substances which may alter tacrolimus whole blood concentrations (see 'Warnings and Precautions' and 'Interactions'). Adjustments to the ADVAGRAF and PROGRAF dose regimen may take several days before steady state is achieved. Most patients can be managed successfully if tacrolimus blood concentrations are maintained below 20 ng/mL. In clinical practice, whole blood trough levels have been 5-20 ng/mL in liver transplant recipients and 10-20 ng/mL in kidney transplant recipients early post-transplant, and 5-15 ng/mL during maintenance therapy. Dose adjustments in specific populations: See SPC. **Contraindications:** Hypersensitivity to tacrolimus or other macrolides or any excipient. **Warnings and Precautions:** Medication errors, including inadvertent, unintentional or unsupervised substitution of immediate-

or prolonged-release tacrolimus formulations, have led to serious adverse events, including graft rejection, or other side effects which could be a consequence of either under- or over-exposure to tacrolimus. Patients should be maintained on a single formulation of tacrolimus with the corresponding daily dosing regimen; alterations in formulation or regimen should only take place under the close supervision of a transplant specialist. ADVAGRAF only limited experience in non-Caucasian patients and those at elevated immunological risk. ADVAGRAF is not recommended for use in children below 18 years due to limited data on safety and efficacy. ADVAGRAF and PROGRAF: During the initial period routinely monitor blood pressure, ECG, neurological and visual status, fasting blood glucose, electrolytes (particularly potassium), liver and renal function tests, haematology parameters, coagulation values, and plasma protein determinations; consider adjusting the immunosuppressive regimen if clinically relevant changes are seen. Monitor tacrolimus levels when co-administering strong inducers or inhibitors of CYP3A4. Herbal preparations, including those containing St. John's Wort, should be avoided. Extra monitoring of tacrolimus concentrations is recommended during episodes of diarrhoea. Avoid concomitant administration of ciclosporin. Ventricular hypertrophy or hypertrophy of the septum (reported as cardiomyopathy) have been reported, occurring with tacrolimus blood trough concentrations much higher than the recommended maximum tacrolimus blood trough concentrations levels. Other risk factors for these conditions include pre-existing heart disease, corticosteroid usage, hypertension, renal or hepatic dysfunction, infections, fluid overload, and oedema. Echocardiography or ECG monitoring pre- and post-transplant is advised in high-risk patients, and dose reduction or a change of immunosuppressive agent should be considered if abnormalities develop. Tacrolimus may prolong the QT interval. Exercise caution in specific patients – see SPC. Patients are at increased risk of all opportunistic infections including BK Virus associated nephropathy and JC Virus associated progressive multifocal leukoencephalopathy (PML); consider in patients with deteriorating renal function or neurological symptoms. Patients have been reported to develop posterior reversible encephalopathy syndrome (PRES), if so radiological tests should be performed. If PRES is diagnosed, control blood pressure and seizures and immediately discontinue tacrolimus. Epstein Barr Virus (EBV)-associated lymphoproliferative disorders have been reported: concomitant use of other immunosuppressives such as antilymphocytic antibodies increase the risk. EBV-Viral Capsid Antigen (VCA)- negative patients have been reported to have increased risk of lymphoproliferative disorders; EBV-VCA serology should be ascertained before starting tacrolimus treatment. During treatment, careful monitoring with EBV-PCR is recommended. Exposure to sunlight and UV light should be limited. The risk of secondary cancer is unknown. Dose reduction may be necessary in patients with severe liver impairment. Cases of pure red cell aplasia (PRCA) have been reported in patients treated with tacrolimus. All patients reported risk factors for PRCA such as parvovirus B19 infection, underlying disease or concomitant medications associated with PRCA. The printing ink used to mark ADVAGRAF capsules contains soya lecithin. In patients who are hypersensitive to peanut or soya, the risk and severity of hypersensitivity should be weighed against the benefit of using ADVAGRAF. Capsules contain lactose. **Interactions:** See SPC. Tacrolimus is metabolised by CYP3A4. Concomitant use of CYP3A4 inhibitors/inducers may increase/decrease tacrolimus blood levels. Monitoring of tacrolimus blood levels, renal function, side effects and QT prolongation is strongly recommended during concomitant use. Interrupt/adjust tacrolimus dose as necessary to maintain similar tacrolimus exposure. Tacrolimus is a CYP3A4 inhibitor; concomitant use with products metabolised by this enzyme may affect the metabolism of these products. **Pregnancy and lactation:** Tacrolimus can be considered in pregnant women when there is no safer alternative. Cases of spontaneous abortion have been reported. In case of *in utero* exposure, monitoring of the newborn for the potential adverse events of tacrolimus is recommended. Women should not breast feed whilst receiving tacrolimus, see SPC. **Undesirable effects:** Infections: Cases of BK Virus associated nephropathy, as well as cases of JC Virus associated PML have been reported. Neoplasms: Increased risk of malignancies. Malignant neoplasms including EBV-associated lymphoproliferative disorders and skin malignancies have been reported. Cases of pure red cell aplasia have been reported. Very Common (≥1/10): Hyperglycaemic conditions, diabetes mellitus, hyperkalaemia, insomnia, tremor, headache, hypertension, diarrhoea, nausea, renal impairment, infections, liver function test abnormal, Common (≥1/100 to <1/10): Haematological abnormalities, electrolytes decreased, fluid overload, hyperuricaemia, appetite decreased, metabolic acidoses, lipid disorders, hypophosphataemia, anxiety symptoms, mental disorders, confusion and disorientation, depression, depressed mood, mood disorders and disturbances, nightmare, hallucination, seizures, disturbances in consciousness, paraesthesias and dysaesthesias, peripheral neuropathies, dizziness, writing impaired, vision blurred, photophobia, eye disorders, tinnitus, ischaemic coronary artery disorders, tachycardia, haemorrhage, thromboembolic and ischaemic events, vascular hypotensive disorders, peripheral vascular disorders, dyspnoea, parenchymal lung disorders, pleural effusion, pharyngitis, cough, nasal congestion and inflammations, gastrointestinal inflammatory conditions, gastrointestinal ulceration and perforation, gastrointestinal haemorrhages, stomatitis, ascites, vomiting, gastrointestinal disorders, bile duct disorders, cholestasis and jaundice, hepatocellular damage and hepatitis, cholangitis, pruritus, rash, alopecias, acne, sweating increased, arthralgia, muscle spasms, limb and back pain, renal failure, oliguria, renal tubular necrosis, nephropathy toxic, urinary abnormalities, bladder and urethral symptoms, asthenic conditions, febrile disorders, pain, discomfort, oedema, blood alkaline phosphatase increased, weight increased, body temperature perception disturbed, primary graft dysfunction. Uncommon (≥1/1000 to <1/100): Coagulopathies, coagulation and bleeding analyses abnormal, pancytopenia, hypoproteinaemia, hyperphosphataemia, hypoglycaemia, dehydration, coma, central nervous system haemorrhages and cerebrovascular accidents, paralysis and paresis, encephalopathy, speech and language disorders, amnesia, cataract, arrhythmias, cardiac arrest, heart failures, cardiomyopathies, ECG investigations abnormal, pulse investigations abnormal, weight decrease, ventricular hypertrophy, palpitations, infarction, deep venous thrombosis, shock, respiratory failures, respiratory tract disorders, asthma, paralytic ileus, peritonitis, acute and chronic pancreatitis, amylase increased, blood lactate dehydrogenase increased, gastrooesophageal reflux disease, impaired gastric emptying, anuria, haemolytic uraemic syndrome, uterine bleeding, psychotic disorder, multi-organ failure. Rare (≥1/10,000 to <1/1000): Thrombotic thrombocytopenic purpura, blindness, neurosensory deafness, pericardial effusion, acute respiratory distress syndrome, subileus, pancreatic pseudocyst, hepatic artery thrombosis, venoocclusive liver disease, toxic epidermal necrolysis (Lyell's syndrome), mobility decreased, fall, ulcer, chest tightness, thirst. Very rare (<1/10,000): ECG abnormal, ECG QT prolonged, *Torsades de Pointes*, hepatic failure, Stevens Johnson syndrome, nephropathy, cystitis haemorrhagic. Not known: Pure red cell aplasia, agranulocytosis, haemolytic anaemia. Consult the SPC for complete information on side effects and full prescribing information. **Packs and prices:** Country-specific. **Legal Classification**: POM. **MA Number**: PROGRAF: Country specific. ADVAGRAF: EU/1/07/387/001-26. **Date of Revision**: November 2015. Further information available from Astellas Pharma Europe Ltd, 2000 Hillswood Drive, Chertsey, Surrey, KT16 0RS, UK. ADVAGRAF and PROGRAF are registered trademarks. ADV/11/0030/EUc(4).

Adverse events should be reported. UK residents: Reporting form and information can be found at www.mhra.gov.uk/yellowcard.
Adverse events should also be reported to Astellas Pharma Ltd. on 0800 783 5018. Non-UK residents: Report adverse events to Astellas Pharma Europe by email to safety-eu@astellas.com, by facsimile to +31 (0)71-545 5208, or contact your local Astellas office (www.astellas.eu/contact/locations/).

CORRECTION NOTICE

This online version of the Supplement to *Transplantation* (Advancing Transplantation: New Questions, New Possibilities in Kidney and Liver Transplantation by Wadström et al), published in February 2017, has been revised from the original published version. The Astellas job code and date of preparation have been updated from 'ADV/16/0009/EU(1)' and 'January 2017', respectively, to 'ADV/16/0009/EU(2)' and 'March 2017', on the front cover and page S1. The 'Prescribing information and adverse event reporting information' on pages S40–S41 has been replaced. Key changes are as follows: The wording on pages S40–S41 has been updated for sections: 'Posology and Administration', 'Prophylaxis of transplant rejection', 'Therapeutic drug monitoring', 'Warnings and Precautions', 'Interactions', 'Pregnancy and lactation', and 'Undesirable effects'. The following text has been added on page S40: 'Increased doses of tacrolimus, supplemental corticosteroid therapy and introduction of short courses of mono-/polyclonal antibodies have all been used. If signs of toxicity are noted the dose may need to reduced. For conversion to PROGRAF, treatment should begin with the initial oral dose recommended for primary immunosuppression', and 'Dose adjustments in specific populations: see SPC.' The following text has been added on page S41: 'MA Number: PROGRAF: Country specific. ADVAGRAF: EU/1/07/387/001-26' and 'ADVAGRAF and PROGRAF are registered trademarks. ADV/11/0030/EUc(4)'. The date of revision for the 'Prescribing information and adverse event reporting information' on page S41 has been changed from 'January 2017' to 'November 2015'. The address on page S40 for obtaining additional information has been changed from 'Astellas Pharma Ltd, 2000 Hillswood Drive, Chertsey, KT16 0RS' to 'Astellas Pharma Europe Ltd, 2000 Hillswood Drive, Chertsey, Surrey, KT16 0RS, UK'.